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5956

Local Formation of Antibody by the Skin.

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One of the basic problems of local tissue-immunity is the relationship of immune bodies to the reaction, Besredka¹ minimizes their importance and defines local immunity as "an immunity without the obligatory participation of antibodies". Gay² takes a broader view in defining the condition as "a locally superior mechanism for the disposal of a particular microorganism" and considers that it may be demonstrated either by the local presence of antibodies before their appearance elsewhere in the body, by their local presence in greater concentration than elsewhere, or by a superior method of direct disposal of bacteria in the particular area in question.

Cannon and Pacheco³ have described the "fixation" of virulent staphylococci in the skin of guinea pigs which had been locally immunized by intradermal injections of a staphylococcal vaccine. This immunization caused thickening of the subcutis by a dense layer of newly formed histiocytic tissue. Living staphylococci, when introduced into such a tissue, remained localized in a small

¹ Besredka, A., Williams and Wilkins, Baltimore, Md., 1927.

² Gay, Frederick P., *The Newer Knowledge of Bacteriology and Immunology*, Jordan, E. O., and Falk, I. S., The University of Chicago Press, 1928, 881.

³ Cannon, Paul R., and Pacheco, G. A., *Am. J. Path.*, 1930, **6**, 749.

area so that, in general, the animals suffered no serious effects. Similar injections into normal guinea pigs, however, led to a cellulitis and the death of many animals from generalized sepsis. Microscopical examinations showed the staphylococci concentrated in clumps and masses in the locally immunized tissues, whereas the microorganisms were diffusely disseminated through the subcutis of the normal animals. The assumption was made that an important element in the mechanism of fixation was an antigen-antibody reaction whereby the bacteria were opsonized and agglutinated by antibodies formed *in loco* by the histiocytes which had been mobilized during the period of local immunization. As a secondary reaction, leucocytes then infiltrated the area and kept the bacteria localized, thus preventing generalization with its serious consequences.

This assumption of agglutination *in vivo* was based solely upon morphological evidence. We have now demonstrated by a method of chemical extraction that antibodies may be present in relatively high concentration in tissues which have been locally immunized, thus confirming the assumption and furnishing a more definite basis for the recognition of local immunity as an important element in the general mechanism of defense.

Rabbits were injected intradermally in a local area of the abdominal wall with a suspension of *B. paratyphosum B* in 0.85% solution of sodium chloride to which 0.2% formalin had been added. This microorganism was chosen merely because of the ease with which its agglutinins can be demonstrated. The injections were made daily for from 5 to 12 days, during which period it was noted that the later injections were usually followed by an accelerated formation of nodules, frequently with ulceration, at the site of injection, thus suggesting an increasing tendency to localization of the vaccine injected. After the termination of the immunization the animals were kept in their cages for from 18 to 22 days and were then, with the exception of rabbits 5 and 6, reinoculated into the locally immunized area with 1 cc. of the original vaccine suspension, in order to cause more antibody to be liberated. Seven to 14 days later, the locally treated tissues and the corresponding untreated tissues of the opposite side, together with liver, spleen, and blood serum, were added to a solution of equal parts of sterile glycerol and 0.85% solution of sodium chloride in the proportion of one part of tissue to 15 parts of the extraction fluid, were ground aseptically with the exception of the serum, and kept under chloroform-toluol at 37°C. for from 6 to 9 days. The solutions were then titrated simultaneously against a suspension of living paratyphoid bacilli in order to

determine the relative concentrations of agglutinin in the various tissues under such comparable conditions. The glycerol-saline extractive was used because Cary⁴ and Motohashi⁵ found it the best of several tried in their study of the site of formation of antibodies. Their work furnished the first convincing evidence that antibodies are products of macrophages.

Results. Tables I and II show the comparative agglutinin titers for 10 animals. As is seen, the locally immunized skin, without exception, contained a higher concentration of agglutinins (from 2 to 4 times) than the corresponding skin of the opposite side (Rabbits 1, 2, 3, 4, 5, 7, and 9). Furthermore, the concentration was usually higher than that of the blood serum and always higher than that of comparable portions of liver and spleen. In one animal, not included in this report, the agglutinins appeared in high concentration within 48 hours after the intradermal injection of the antigen, at which time they were undetectable in the spleen, kidney and blood serum. Finally, rabbits immunized intravenously (rabbits 6, 8, and 10) with the same quantities of antigen introduced locally into Rabbits 5, 7, and 9, showed a higher concentration of agglutinin in the liver, spleen or both in 2 of the 3 pairs here reported and in 2 additional pairs not included in this paper because of lack of space.

These results indicate that the local injection of antigen into previously mobilized histiocytic tissue may lead to the local formation and retention of immune bodies in relatively high concentration, from which tissue they may diffuse and be recovered in lower concentration in organs elsewhere. These findings substantiate the view

TABLE I.
Comparative Agglutinin-Titer of Tissues from Rabbits Locally Immunized with *B. paratyphosum* B.

Rabbit	1		2		3		4		S	Sp
	u	i	u	i	u	i	u	i		
Tissue*	Sk	Sk	Sk	Sk	Sk	Sk	Sk	Sk		
3840					0	0	0	0	0	0
1920	tr	++	0	tr	0	tr	0	0	0	0
960	+	++	0	+	0	+	0	tr	tr	0
480	++	++	tr	++	tr	++	+	++	+	0
240	++	++	+	++	+	++	++	++	+	0
120	tr	++	+	+	+	++	+	++	++	tr
60	tr	+	tr	tr	tr	+	+	++	++	tr
30	tr	tr	0	0	tr	+	tr	tr	tr	tr

++ strong agglutination. + moderate agglutination. tr trace of agglutination.

*u/Sk skin unimmunized side. i/Sk skin of locally immunized side. Sp spleen. S serum.

⁴ Cary, William E., *J. Med. Res.*, 1922, **43**, 399.

⁵ Motohashi, S., *J. Med. Res.*, 1922, **43**, 419.

TABLE II.
Comparative Agglutinin-Titer of Tissues from Rabbits Locally and Intravenously Immunized with Equal Quantities of *B. paratyphosum* B.

Rabbit Mode of Immun- ization* Tissue	5			6			7			8			9			10		
	Local			Intravenous			Local			Intravenous			Local			Intravenous		
	u	i	Sk	Sk	Sp	S	u	i	Sk	Sk	Sp	S	u	i	Sk	Sk	Sp	S
3840	0	0	0	0	0	0	0	tr	+	+	+	+	0	0	0	0	0	0
1920	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
960	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
480	tr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
240	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
120	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60	tr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	tr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

tr trace of agglutination.

* u/Sk skin of unimmunized side, i/Sk skin of locally immunized side, Sp spleen, L liver, S blood serum.

that a locally increased resistance to infection may be due in part to a locally increased formation of antibodies. Therefore, local immunization of organs and tissues would seem to be indicated, whenever practically possible, as a supplement to the methods of general immunization as practiced at present.

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The Greenish Discoloration Produced on Blood Agar by the Growth of *Pneumococcus*.

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From the Bacteriological Laboratories of the Chicago Medical School.

The fact that the pneumococcus, when grown on media containing blood pigment, produces a greenish disintegration product of hemoglobin, has long been utilized as one of the means of identifying this organism. Jensen¹ states, "Pneumococci and certain streptococci produce a green pigment when grown in blood agar, and this pigment is termed methemoglobin in all text books on bacteriology. Unfortunately the literature of blood pigments is in a state far from clarified. Many European investigators do not attempt to name the particular form of blood pigment, but refer to it simply as 'a green discoloration'."

Since the work of Conant² and Conant and Feiser³ methemoglobin has been characterized as oxidized or monoelectronated reduced hemoglobin. It differs from hemoglobin only in having its iron in ferric form. Its color is definitely brownish without any element of green, and in that manner differs from the color of green hemolysis. Because of this discrepancy, an investigation was undertaken to determine the exact status of the green pigment.

Experimental. The green portions produced in pneumococcus cultures on blood agar were excised and tested for methemoglobin spectroscopically, by ammonium hydroxide, and by sodium cyanide. The differences in the effect produced by the green pigment and by methemoglobin are tabulated:

Methemoglobin	Green Pigment
Characteristic absorption band in the red end of the spectrum	No definite absorption bands
Ammonium hydroxide caused the appearance of reddish alkaline methemoglobin	Caused the appearance of a lemon yellow color
The addition of sodium cyanide solution (0.1%) led to the formation of orange cyanmethemoglobin	Produced a coloration which did not differ from that following the use of ammonium hydroxide (probable alkali effect)

It appears therefore that the green pigment differs radically from

¹ Jensen, L. B., *Bull. Inst. Am. Meat Packers*, 1929, 4, Univ. of Chicago.

² Conant, J. B., *J. Biol. Chem.*, 1923, **57**, 401.

³ Conant, J. B., and Feiser, L., *J. Biol. Chem.*, 1924, **62**, 595.

methemoglobin. Although Neill and Hastings⁴ studied the formation of methemoglobin from hemoglobin by the action of soluble extracts of pneumococcus, this does not seem to be a feature of their growth in culture. For this reason, it was decided to determine why methemoglobin production does not take place under these circumstances. It was decided to utilize the observations of McLeod and Gordon⁵ that pneumococcus in culture produces hydrogen peroxide. The effects of hydrogen peroxide on the blood pigment were studied.

The addition of hydrogen peroxide to a solution of oxyhemoglobin (prepared by laking human red blood cells with distilled water) which is at, or near its isoelectric point decolorizes it, turning it first brown and then a yellowish white. Is this brownish intermediary methemoglobin? A consideration of the relationship submitted by Conant³ would lead us to believe not. Methemoglobin is formed only by oxidation of reduced hemoglobin (Conant³). In the presence of hydrogen peroxide, which constantly liberates molecular oxygen, little if any of the methemoglobin precursor could exist as such.

Addition of ammonium hydroxide to the reaction mixture did not cause the formation of alkaline methemoglobin nor did sodium cyanide give the characteristic color reaction that it develops with methemoglobin. Examination of the reaction spectroscopically reveals that the bands of oxyhemoglobin gradually fade and finally disappear, without the appearance of new absorption bands in the visible spectrum.

To test further the possibility of methemoglobin being an intermediary in the oxidation of blood pigment by peroxide, hydrogen peroxide was added to a solution of methemoglobin prepared by the action of ferricyanide on hemoglobin. A preliminary formation of oxyhemoglobin took place and the reaction proceeded as above. Two other samples of methemoglobin, one prepared by the action of quinone on hemoglobin and one prepared by allowing a solution of oxyhemoglobin to undergo spontaneous denaturation at room temperature, gave the same results.

(The oxyhemoglobin prepared from methemoglobin by the action of hydrogen peroxide shows a definite catalase activity.)

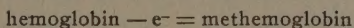
From the foregoing, it is evident that provided pneumococcus produces hydrogen peroxide in culture, there is no possibility for the production of methemoglobin, inasmuch as the latter cannot exist in the presence of the former.

⁴ Neill, J. M., and Hastings, A. B., *J. Biol. Chem.*, 1925, **63**, 487.

⁵ McLeod, J. W., and Gordon, J., *J. Path. Bact.*, 1923, **26**, 326.

The action of hydrogen peroxide on hemoglobin produces a substance which may be the end product of alpha hemolysis. It is assumed to be an oxidation of the unsaturated pyrrols in the prosthetic group. Such an action would furnish an interpretation of the disappearance of the absorption band without detachment of iron from the molecule (free iron could not be demonstrated in the reaction product of peroxide on hemoglobin) the pyrrol nucleus, itself, being chromatophoric as in hematoporphyrin.

That methemoglobin should be unable to exist in solutions containing hydrogen peroxide may be deduced from certain considerations. As demonstrated by Ray and Isaacs⁶ the change from methemoglobin to oxyhemoglobin, brought about by tissue juices or by ferrous ion, in the presence of oxygen, is a consideration following the exposition of the reversible oxidation reduction system:



The transformation is rendered clear if we formulate this system

$$E_1 = E_0 - \frac{RT}{nF} \log_e \frac{(\text{Hb}^{+++})}{(\text{Hb}^{++})} \quad (1)$$

in the presence of oxygen (Hb^{++}) is governed by the equation

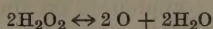
$$(\text{Hb}^{++}) = \frac{K_{\text{HbO}_2}}{(\text{O}_2)} \quad (2)$$

substituting:

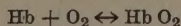
$$E_1 - E_0 - \frac{RT}{nF} \log_e \frac{(\text{Hb}^{+++}) (\text{O}_2)}{K_{\text{HbO}_2}} \quad (3)$$

Since at atmospheric pressures, hemoglobin is practically completely saturated with oxygen, and since at any E_1 some quantity of reduced hemoglobin must exist in solutions of methemoglobin, the equilibrium is disturbed by saturation of the solution with oxygen. In this manner the conversion of methemoglobin to ferrous hemoglobin is accomplished by reducing agents, which we would ordinarily term 'weak' ones, provided oxygen is present.

The oxidation reduction potential of the system



lies in the vicinity of 0.150 millivolts on the saturated calomel scale (unpublished results). The activity of nascent oxygen, as a reducing agent, is evinced only below this potential. However, a side reaction, which participates in the reduction of methemoglobin by nascent oxygen



⁶ Ray, G. B., and Isaacs, L., *Am. J. Physiol.*, 1930, **91**, 377.

apparently serves to raise the equilibrium potential of the methemoglobin-hemoglobin system above this point.

Hydrogen peroxide slowly decolorizes cyanhemoglobin, whereas, when allowed to act on nitrite hemoglobin, the end product is green. This greenish color and its striking resemblance to the pneumococcus discoloration of blood, led us to investigate the nature of this substance.

Pneumococcus cultures were therefore tested for nitrite by the phenylbenzidine reaction with negative results. However, nitrite is not the only compound which, along with peroxide and hemoglobin gives the green color. Nitrites, hydroxylamine and hydrazine were also found capable of converting hemoglobin to this modification. In all probability, some oxide of nitrogen is concerned, the probability being strengthened by the fact that the reaction will not develop in alkaline solutions.

Next we attempted to elicit whether the reaction was due to an action of this nitrogen and peroxide compound on the hematin or the globin portions of the hemoglobin molecule. Experiments with pure hemin were negative. Other proteins, however, gave definite reactions, among these being casein, egg albumin and serum albumin. The fact that in these cases the color was slightly more yellowish than the reaction with hemoglobin but that on alkalination they would turn the same lemon color, leads us to believe that we are here dealing with an xanthoproteic reaction and that this reaction is dependent (as is the xanthoproteic reaction utilizing nitric acid) on the type of protein employed. In fact the xanthoproteic reaction of hemoglobin and nitric acid has a definitely greenish cast.

Summary and Conclusions. 1. Methemoglobin will not exist in the presence of hydrogen peroxide. Therefore the possibility that the green disintegration product of hemoglobin, produced by the growth of pneumococcus is highly remote. 2. A green pigment, identical in all respects with that produced by the growth of pneumococcus on hemoglobin, has been prepared by the action of peroxide and certain nitrogen compounds on the blood pigment. 3. A chemical study of this artificial green pigment indicates that it is not methemoglobin but an xanthoproteic compound.

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Glucose Utilization by Brain.

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Medium-sized dogs were lightly anesthetized by intraperitoneal injection of 60 mg. per kilo of sodium amytal. Occasionally small amounts of ether were used during the operation. The femoral artery and vein and sagittal sinus were exposed and blood samples drawn practically simultaneously from the 3 vessels at half-hour intervals. For 2 to 2½ hours the dog was allowed to lie quietly and was kept warm, with no further treatment. After this time, 10 or 20 units of insulin were given intravenously, and blood samples taken as before for another 3-5 hours. Sugar (reducing substance) was determined by a slightly modified Shaffer-Hartman method after deproteinizing with the usual tungstic acid reagent, duplicate analyses agreeing nearly always. As run, with precautions against oxidation of the reagent by dissolved oxygen, the titration with 0.005 N sodium thiosulphate was entirely linear for known glucose solutions from quantities of 0.05 mg. or less to over 0.6 mg. Glucose added to blood was regularly recovered. In actual determinations, a blank and 2 or more known glucose solutions were included in the series and the blood values determined by interpolation.

Average results of 14 complete experiments are given in Table I, and a single experiment illustrated in Figure I. The animals were not in the post-absorptive state, so that the venous values, over

TABLE I.

Time in hours	Blood Sugar, mg. %		
	Artery (Femoral)	Artery— femoral vein	Artery—sagittal sinus
0.5	144	27	49
1.0	140	25	50
1.5	135	18	38
2.0	130	18	34
2.5 (Insulin injected)	128	17	35
3.0	130	21	48
3.5	121	15	38
4.0	101	17	32
4.5	56	6	12
5.0	44	5	13
5.5	75	5	13
6.0	75	15	25
6.5	90	25	30
7.0	95	20	30

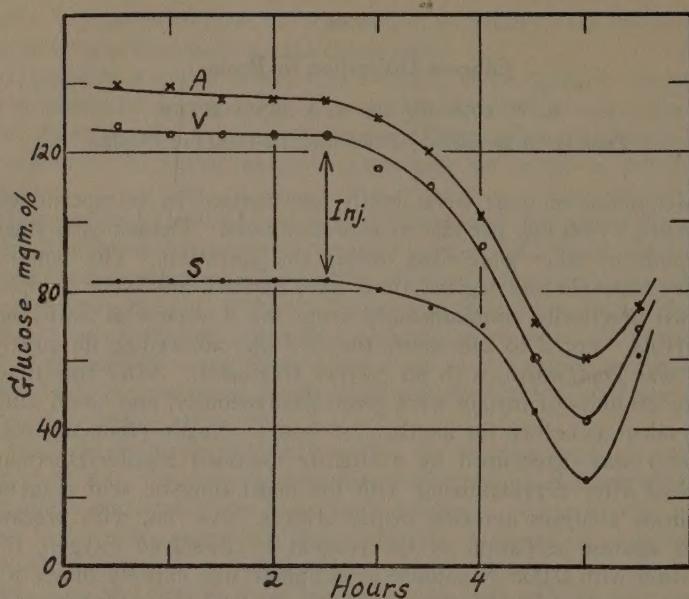


FIG. 1.

March 1, 1930. Dog, 11.2 K. Sodium amytal, 40 mg. per kilo intraperitoneally. First sample 10:05 a. m. Insulin, 10 units, intravenously at $\uparrow$

- x—x Arterial blood sugar (femoral).
- o—o Venous blood sugar (femoral).
- Sinus blood sugar (sagittal).

110 mg. %, are not high. The arterio-venous difference of 17 mg. % just before insulin injection is greater than usually obtained in the post-absorptive state,⁸ but is well within the range for animals after administration of glucose. The sinus blood sugar was consistently, in every experiment, lower than the systemic venous blood and averaged, just before insulin, 35 mg. % below the arterial.

Following insulin, all sugar values fell to a low point in 2½ hours and then slowly returned towards normal. At the low point of the curve, venous blood sugar averaged 39 mg. % and the arterio-venous and arterio-sinus differences were much diminished, respectively to 5 and 13 mg. %. Although the arterio-venous difference has been shown in starved animals to be greater under insulin than in controls,^{1, 11} the present contrary findings on fed animals are not necessarily in conflict. Schmidt's¹¹ findings on dogs under ether do

¹ Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

show, in fact, lesser arterio-venous sugar differences as the blood sugar falls under insulin than before. The early rise in arterial values was not present in the best experiments.

Although we have not determined blood flow ourselves, many data^{2, 7, 8, 12} indicate a normal flow of 140 cc. per minute per 100 gm. brain tissue, which is cut to about 50 in moderate narcosis. For the leg, the equivalent figure is 5 cc.⁸ Calculating the actual amount of sugar taken up by the anesthetized brain and the leg respectively, the values are 10.5 and 0.5 mg. per gm. per hour. If all this were completely oxidized, it would correspond to 8 cc. of oxygen used per gram per hour for brain and 0.4 cc. for the leg. The actual oxygen consumption of dog brain *in situ*, as determined by Gayda,² is 6, by Schmidt,¹⁰ 9, for the unanesthetized animal. Comparison of the 2 sets of data indicates that all the sugar reaching the brain from the blood might be oxidized. This is in conformity with the finding of an R.Q. of 1 for the brain,^{2, 5, 10} and with the relative constancy in the quantity of brain glycogen.⁶ Also, McGinty⁹ has found less lactic acid in the sagittal sinus blood than in arterial when asphyxial conditions are avoided. On the other hand, the sugar values are somewhat high compared to the oxygen consumption, so that small amounts might have been deposited as glycogen, changed to lactic acid, etc.

The sugar uptake by the extremity is also somewhat higher than the measured oxygen consumption. Himwich and Castle⁴ found 0.36 cc. of oxygen taken up by resting muscle, and the value for the leg as a whole must be lower. It seems certain in this case that a considerable portion of the glucose disappearing is deposited as glycogen or changed to lactic acid.

Summary. < The metabolism of the brain, as estimated by the glucose taken from blood in passing through it, is in agreement with that determined by analogous studies of oxygen and carbon dioxide

² Gayda, T., *Arch. di Fisiol.*, 1914, **12**, 214.

³ Henriques, V., and Ege, R., *Biochem. Z.*, 1921, **119**, 121.

⁴ Himwich, H. E., and Castle, W. B., *Am. J. Physiol.*, 1927, **83**, 92.

⁵ Himwich, H. E., and Nahum, L. H., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 496.

⁶ Holmes, E. G., and Holmes, G. E., *Biochem. J.*, 1926, **20**, 1196.

⁷ Hou, C. L., *J. Orient. Med.*, 1926, **5**, 20.

⁸ Jensen, P., *Pflüger's Arch.*, 1904, **103**, 171.

⁹ McGinty, D. A., *Am. J. Physiol.*, 1928, **85**, 395.

¹⁰ Schmidt, C. F., *Am. J. Physiol.*, 1928, **84**, 223.

¹¹ Schmidt, V. A. A., *Arch. exp. Path. u. Pharm.*, 1930, **153**, 79.

¹² Winterstein, H., *Handb. d. norm. u. path. Physiol.*, 1929, **9**, 515.

changes. Brain metabolism so estimated is at least 20 times as intense as that of resting muscle. This average does not differentiate the higher respiration of gray matter from white, the large amount of non-neural elements, and similar factors tending to dilute a much higher value for the nerve cells proper. Insulin does not uniquely influence brain sugar utilization.

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Effect of Calcium Administration to Rachitic Rabbits.

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The rabbits used in this work were made rachitic with the McCollum diet 3143, which is high in calcium and low in phosphorus. The calcium is present, chiefly, in the form of calcium carbonate. Previous work with this diet has shown that after about 20 days the animals have more or less marked rickets, as evidenced by X-ray of the bones and by determination of the phosphorus in the serum.

The control animals were given the same diet with the addition of cod liver oil, about 1 cc. per 100 gm. of diet.

Calcium was given in the form of calcium chloride or calcium gluconate, both in the strength of 10 mg. Ca per cc. of solution, administered in one dose by stomach tube. The dose, both in controls and rachitic animals, varied from 25 mg. to 200 mg.

Blood was taken by heart puncture before the administration of calcium and again a few minutes afterwards.

The normal animals did not seem affected by the administration of calcium. Of the 24 rachitic animals, however, 12 died within 15 minutes of the administration. In these animals the blood was very thick and coagulated rapidly.

The cause of death in the rachitic animals was, probably, hypercalcemia. Before the administration of calcium both controls and rachitic animals showed, rather constantly, a serum calcium of about 12 mg. per 100 cc. After the administration of calcium one control animal showed a rise of the serum calcium to 19 mg., in the remaining controls the maximum rise was to 15 mg. In the rachitic animals quite a number showed extremely high values for serum calcium after calcium had been given by mouth. The maximum was 61 mg.,

and 12 of the 24 showed values of 20 mg. or higher.* These were the 12 animals who died immediately after the administration.

The serum phosphorus was, generally, not affected by these very marked increases in serum calcium.

The increase in serum calcium was, roughly, correlated to the dose given, although in some cases a marked increase of serum calcium was obtained after the administration of only a small amount of calcium by mouth. There seemed to be no difference in the effect of the gluconate and the chloride.

As it seems possible that the above results may be due to hyperparathyroidism, we are now determining the amounts of parathyroid hormone in the serum of normal and rachitic rabbits.

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Formation of Antibodies in Fertile Hens Eggs.

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From the Department of Research Bacteriology, Northwestern University Medical School.

The theoretical and practical interest inherent in the question of the time and location of antibody formation in the developing organism is responsible for a series of experiments in which fertile hens' eggs were repeatedly injected with foreign sera. After incubation for a certain time, the eggs were opened and precipitin tests were made on the remaining egg white. These tests were positive in approximately 4% of the cases.*

The handling of the eggs during the process is quite delicate, and in spite of careful manipulation, a number of eggs were lost. Because of the obvious necessity of insuring a well-growing chick, the best grade of hatching eggs must be procured. The first injection is usually made on the third day of incubation, when fertility may be ascertained. The egg shell was first washed with mercuric bichloride and then with alcohol. Into each egg, from 0.1 to 0.25 cc. of sheep serum [foreign soluble protein] which had previously been passed through a Berkefeld filter, was injected. In piercing the shell, care must be taken to cause as little disturbance of the con-

* 5 animals had a serum Ca of 20-29, 5 of 30-39, and 2 over 40.

* Young animals are poor antibody producers. R. H. Jaffé, *Physiol. Rev.*, 1931, 11, 279.

tents as possible. The same precaution must be observed while forcing the foreign protein out of the syringe. After the injection had been made, the needle was retracted, and the hole immediately sealed with fairly thick collodion. These injections were repeated 3 or 4 times at intervals of 2 to 3 days, and the eggs were continuously incubated at 37.5°C , and handled as they would be for ordinary hatching purposes. After a total of 14 days' incubation, the eggs were opened and the egg white removed. It was diluted with 5 volumes of physiological salt solution, and filtered until perfectly clear.

Precipitin tests were made in two series: (1) the egg white concentration was kept constant and the antigen diluted, (2) the egg white was diluted and the antigen concentration kept constant. All dilutions were made with physiological salt solution. Controls included normal egg white, serum of another species, and hypo- and hypertonic salt solutions. From 126 fertile eggs successfully handled in this manner, 5 gave positive precipitin tests. The highest titre observed was 1 : 500 [antigen diluted], and the lowest 1 : 50.

Attempts made to hatch chicks from eggs repeatedly injected with foreign proteins were found to be successful in a number of cases, particularly when the injections were begun at a later stage of incubation. As an insufficient number of such animals have been tested up to the present time, no definite statements may be made, but it was observed that antibody formation was apparently obtained in a low percentage. The same holds true for experiments of passive transfer into other test animals of egg white in which the presence of antibody had been previously demonstrated.

A general discussion of the results must be postponed. One minor aspect deserves mention. That antibodies can be produced offers support to the conception that the egg contents are at least partly or as a first step absorbed before utilization by the embryo. The presence of antibodies in the remaining egg white may be attributed either to permeability of the vessels concerned, or to the difficulty of separating the egg white proper from the circulatory channels and their blood content.

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Manometric Studies on the Bio-oxidation of Histamine.

E. GEBAUER-FUELNEGG AND HOWARD L. ALT.

(Introduced by C. J. Farmer.)

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Northwestern University Medical School.*

Best and McHenry¹ reported observations on a histamine-inactivating substance or system which they denote as histaminase. They state that oxygen is consumed during this reaction but have postponed a detailed manometric study until obtaining a more concentrated histaminase preparation. We have found that a kidney powder, prepared according to their directions, when added to histamine gave sufficient gas changes to study the histamine-histaminase reaction from the standpoint of oxygen uptake.

The experiments were performed in the Barcroft-Warburg apparatus, with air in the gas space, phosphate buffer in the conical vessel, KOH in the well, and a measured amount of histamine in the side bulb. Since attempts at materially purifying the enzyme were unsuccessful, 200 mg. dry kidney powder from the dog were used in the phosphate solution as a source of histaminase. When the system was at a constant temperature, 37.5° C., the histamine was tipped into the vessel and manometric readings were made at regular intervals until the oxidation ran to completion. Parallel experiments were always made without histamine because the powder alone had a small oxygen uptake. (Ca 25% of that with histamine.) This was subtracted in every case from the values found with the addition of the base.

The rate of oxidation early in the experiments was constant. Ten minute observations of the histamine oxidation plus the gas change due to kidney powder averaged about 6 to 8 cmm. of oxygen. When the oxygen uptake had ceased, the histamine was inactivated as shown by its failure to contract guinea pig intestine *in vitro*. On the other hand, when the oxygen uptake was not complete, the substrate was capable of contracting smooth muscle. This was observed in several experiments. It was thought at first that an indication of the nature of the end products of the reaction could be obtained from the total amount of oxygen necessary to inactivate a given amount of histamine. This was not found to be the case. The first lot of kidney powder consumed an average of 41 cmm. oxygen per 0.5 mg.

¹ Best, C. H., and McHenry, E. W., *J. Phys.*, 1930, **70**, 349.

histamine dihydrochloride (neutralized) whereas the second lot consumed an average of 85 cmm. for an equal quantity. In the first case, the oxygen uptake with 0.5 mg. histamine was complete in 2 hours, while in the second, it required the same length of time for 0.25 mg. Apparently in neither case can the molar ratio between the amount of histamine inactivated and the total oxygen consumed be expressed in whole numbers.* It is possible that these discrepancies can be explained by concomitant or functional reactions not due to the histamine-histaminase oxidation or by varying degrees of inhibition by an unknown factor.

In connection with the question of the chemical fate of histamine, it was found that no CO_2 was given off during the reaction, *i. e.*, the respiratory quotient was zero. Furthermore there was no oxygen uptake when iminazole aldehyde, iminazole lactic acid, iminazole propionic acid, and histidine were added to active kidney powder.

When the kidney powder was kept constant at 200 mg., the rate of oxidation was independent of amounts of histamine varying from 0.25 to 2 mg., whereas the total oxygen uptake was directly proportional to these quantities. When the amount of histamine was kept constant at 1 mg., the rate of oxidation was roughly proportional to amounts of kidney powder, varying from 50 to 300 mg., whereas the total oxygen uptake was constant. At 9°C . there was no oxygen consumption in the histamine-histaminase system; at 55°C . the reaction was markedly accelerated, being complete in approximately one-third of the time required at 37.5°C . The rate of oxidation was the same when a buffer of pH 5.2, 7.4, and 10 was used. However, the total oxygen uptake of 0.5 mg. of histamine was 57.8 cmm., 87.4 and 127.5 cmm. for these respective buffers. A repetition of the experiment with 0.25 mg. histamine gave results of 30, 42.2, and 66.7 cmm. oxygen for the same pH concentrations. The histamine was inactivated in every instance after the oxygen consumption had ceased, as shown by its failure to contract smooth muscle.

Potassium cyanide, 0.002 M and 0.0002 M, inhibited the rate of oxidation 60% and 25% respectively during the first hour but had no influence on the total oxygen uptake. The histamine-histaminase reaction as revealed by the gas change was not influenced by either a 95% CO -5% O_2 mixture or by a 0.1 molar Na Pyrophosphate solution. This excludes certain forms of iron catalysis.

* Bernheim² has reported a simple molar relationship in the case of the tyramine-tyraminase reaction.

² Bernheim, M. L. C., *J. Biol. Chem.*, 1931, **93**, 299.

A preparation made from the small intestine of the dog contained almost as much histaminase as did kidney powder, while analogous preparations from the brain, spleen, and lung showed no oxygen uptake with histamine after 3 to 4 hours' observation. Kidney powder from the guinea pig, cat, human (obtained 2 hours after death), rat, and chicken with histamine showed such slight oxygen consumption that they were not adequate as a source of histaminase in these experiments. Fresh dog kidney in the form of a 200 mg. chunk showed about a 50% increase in oxygen consumption when 1 mg. of histamine was added. Freshly ground up kidney gave a much smaller histaminase effect.

An attempt to split histamine on a blood-charcoal model with the uptake of oxygen was unsuccessful.† Histidine, however, had a definite oxygen uptake on blood charcoal in phosphate buffer solution. Warburg and Negelein³ in a similar type of experiment have observed the splitting of cystine, leucine, and tyrosine with an uptake of oxygen.

The manometric measurements reported above agree with the physiological and chemical observations of Best and McHenry in most respects. However, they observed (1) decreased inactivation of histamine at pH 10, (2) that the optimum inactivation of histamine occurred at 37° with a destruction of the inactivating enzyme at 60°, and (3) complete inhibition of the inactivating substance with KCN at 0.004, 0.002, 0.001, and 0.0005 molar concentrations.

5962

Reactions of Proteins in Liquid Ammonia with Metallic Sodium.

CLEMMY O. MILLER AND RICHARD G. ROBERTS.

(Introduced by C. J. Farmer.)

From the Department of Chemistry, Northwestern University Medical School.

McChesney and Miller¹ introduced the use of liquid ammonia as a medium for the study of the chemical properties of proteins. They found that proteins were acidic in liquid ammonia and that when they were treated with alkali metals at -33.5° C. or with ammono

† Choline, acetylcholine, neurine, tyramine, as well as betaine, showed no gas change with the charcoal model.

³ Warburg, O., and Negelein, E., *Biochem. Z.*, 1921, **113**, 257.

¹ McChesney and Miller, *J. Am. Chem. Soc.* (1931), **53**, 3888.

bases at 80-120° C. for 48-72 hours, they are partially ammonolyzed. We have reported² a study of the liberation of hydrogen when sodium is added to liquid ammonia solutions or suspensions of silk fibroin, diketopiperazine, glycyl-alanine, n-methylacetamide, glycollic acid, acetanilide, tyrosine, and alanine. This report includes the results of further investigation on silk fibroin, diketopiperazine, and dipeptides, and investigations on casein, egg albumin and edestin. A chemical examination of the products formed will be carried out later.

When the quantity of hydrogen liberated by a sample of protein, such as silk-fibroin* and egg albumin having a definite nitrogen content is plotted against the quantity of sodium added, the curve may be divided into 3 distinct parts.

The first part of the curve is characterized either by absorption of sodium by the protein or by the efficient absorption of hydrogen by one part of the protein molecule following the liberation of hydrogen by sodium from another part of the molecule, or both. Any of these events involves reduction of some part of the protein molecule. When 2 sodium atoms are added for each 4 nitrogen atoms, the reduction is equivalent to the addition of 2 hydrogen atoms. The second part of the curve covers a range in which the quantity of hydrogen liberated approximately parallels the quantity of sodium added. The third part of the curve is characterized by the condition where all of the available hydrogen is liberated by the sodium and the protein is saturated with respect to the sodium. The point where the second and third parts of the curve join represents a condition in which 5 sodium atoms have been added for each 4 atoms of nitrogen in the sample and 2 atoms of hydrogen have been formed. The total extent of the reduction of the protein is equivalent to the absorption of 3 hydrogens for 4 atoms of nitrogen. While the curves for silk fibroin and casein are not identical, they have the same general form. Our data for egg albumin and edestin indicate that their curves will have a similar form.

These observations raise 2 questions: (1) What is the origin of the hydrogen that is liberated? and (2) What part of the protein molecule is reduced? The carboxyl groups in a protein molecule are acidic and would be one origin of hydrogen, but they are of minor importance, for the acidity of silk fibroin does not indicate

² Presented before the Organic Division, Am. Chem. Soc., Indianapolis, March, 1931.

*The silk fibroin was furnished to us by Cheney Brothers, South Manchester, Connecticut.

that there are two carboxyl groups for each 4 nitrogen atoms present. Since certain compounds containing the imide group are acidic in liquid ammonia, as for example *n*-methyl acetamide, it would suggest the possibility of the imide link in the protein or the diketopiperazine rings, if they are present, as the origin of the hydrogen. An investigation of the acid properties of glycylalanine and glycylglycine has shown that the imide links of these dipeptides are not acidic. Whether or not the imide links in a longer polypeptide chain would be acidic in liquid ammonia or more readily reduced is not known and must be determined. In the case of diketopiperazine, two molecules of diketopiperazine containing 4 imide links will decolorize a solution containing 4 atoms of sodium with the liberation of 0.9 of an atom of hydrogen. Diketopiperazine is, therefore, reduced by the sodium. When an equimolecular mixture of diketopiperazine and glycylglycine is treated with sodium, there is no vigorous reaction of the sodium with the hydrogen of the carboxyl group. For each 4 atoms of nitrogen in the mixture, 4 sodiums react to give one-fourth of an atom of hydrogen or less. If proteins contain diketopiperazine rings as Abderhalden has suggested, then they would be the part of the protein molecule that is reduced by the sodium.

More experimental work must be carried out before we can state definitely what part of the protein molecule furnishes the hydrogen and what part of the molecule is reduced.

5963

Some Properties and Reactions of Carbohydrates in Liquid Ammonia.

CLEMMY O. MILLER AND ARTHUR E. SIEHRS.

(Introduced by C. J. Farmer.)

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An accurate insight into the intermediary metabolism of carbohydrates is dependent in part upon a knowledge of the structure of glycogen and blood sugar. Water has been and still is the most frequently used medium for the study of the chemical properties of the carbohydrates, particularly of the sugars. It occurred to us that liquid ammonia might also be a solvent for the sugars and a medium for the study of the chemical properties of the polysaccharides. Schmid

and his collaborators^{1, 2, 3, 4} have studied some of the physical properties of the polysaccharides in liquid ammonia and have prepared a few of the alkali metal salts of them.

The monosaccharides, arabinose, glucose, levulose, and galactose; the disaccharides, sucrose, maltose and lactose; and the hexoside, α -methylglucoside, are readily soluble in liquid ammonia at -33.5°C . and at 25°C . Glucose is soluble to the extent of 40 gm. per cc. Maltose is the least soluble of the sugars mentioned. α -methylglucoside is soluble to the extent of 36 gm. per 100 cc. Glycogen is dispersed in liquid ammonia to give an opalescent solution. Liquid ammonia solutions of sugars that have been thoroughly dried from water and placed in sealed glass tubes show no caramelization even after standing for a year in the diffuse light of the laboratory. However, caramelization takes place readily when a small quantity of moisture is present. When the ammonia is allowed to evaporate from these solutions, the monosaccharides remain as very thick syrups, which dry in a vacuum desiccator over sulphuric acid to hard glassy substances. Even in this condition, they still contain some ammonia. They do not crystallize from their ammonia solutions. Various attempts to induce crystallization failed. The disaccharides and α -methylglucoside crystallize from their ammonia solutions during concentration. The crystalline masses contain varying quantities of ammonia which is removed with considerable difficulty. For example, a sucrose still contains $2\frac{1}{2}\%$ of ammonia after standing one week in a vacuum desiccator over sulphuric acid.

When solutions of potassium amide are combined with fairly saturated solutions of monosaccharides, disaccharides and α -methylglucosides at -33.5°C . their white potassium salts precipitate immediately. The mono-potassium salts of the monosaccharides, arabinose, glucose, and levulose, are formed; the di-potassium salts of the disaccharides, sucrose and lactose; and either the mono- or tri-potassium salt of α -methylglucoside depending upon the proportions of the potassium amide and glucoside used. There is no caramelization. The salts contain a varying amount of ammonia which is removed with difficulty. The mono-potassium salts of arabinose, glucose, and fructose when wet with ammonia turn brown and char rapidly on exposure to air, but are stable towards air when thor-

¹ Schmid and Becker, *Ber.*, 1925, **53B**, 1968.

² Schmid and Bilowitzki, *Ber.*, 1927, **47**, 785.

³ Schmid, Ludwig and Pretsch, *Ber.*, 1928, **49**, 118.

⁴ Schmid and Zacherl, *Monatsch.*, 1929, **53**, **54**, 498.

oughly dried from ammonia. The potassium salt of maltose chars in air in a few seconds even when dry. The mono-potassium salt of α -methylglucoside is stable toward air. The tri-potassium salt is stable toward air when dry but chars readily when wet with ammonia.

The sugars were recovered from all of these salts and identified by appropriate tests.

When glycogen is suspended in liquid ammonia and a solution of potassium amide is combined with it at -33.5°C ., a white precipitate separates out immediately. This precipitate chars immediately upon exposure to air, even when dry. This indicates that glycogen is precipitated as a salt and not as a result of the neutralization of the electric charge on the glycogen micelles.

When the reducing sugars, arabinose, glucose, levulose, galactose, maltose, and lactose, are sealed in glass tubes with sodamide and allowed to stand at 20°C ., caramelization takes place in less than 24 hours. This is analogous to the action of strong bases on the reducing sugars. Sucrose does not caramelize under these conditions.

Our interest in the alkali salts of the carbohydrates lies in the fact that they may be of use in the preparation of esters and ethers of these substances. Preliminary tests have justified this interest.

5964

Bronchial Stenosis in Suppurative Pulmonary Lesions.

W. E. ADAMS. (Introduced by Edmund Andrews.)

From the Department of Surgery, University of Chicago

Suppurative lesions of the lungs, as well as in other parts of the body, are usually treated by securing proper drainage and rest of the part. One of the most common methods of obtaining these requisites is massive collapse of the involved region. A procedure for producing experimental massive atelectasis has recently been developed in these laboratories.¹ Briefly, this consists of the production of complete stenosis of a bronchus by cauterizing its mucous lining with a 35% solution of silver nitrate. Two weeks following the application of the cautery, the bronchial lumen is found completely occluded and is accompanied by massive atelectasis of the

¹ Adams, W. E., and Livingstone, H. M., *Ann. Surg.*, 1932, **59**, 106.

obstructed lung. This method of producing massive atelectasis secures absolute rest of the part and shuts off the exit draining the lesion. It was determined to test the effect of massive atelectasis on abscess of the lung produced experimentally. Lung abscesses were produced in dogs by the Cutler-Holman technique. In 8 dogs emboli were introduced containing mixed pyogenic organisms. In a second group of 12 dogs, emboli containing human tubercle bacilli were lodged in the pulmonary lobes. Cauterization of the bronchi of the embolized lobes was performed immediately following embolization. Two weeks later bronchoscopy was repeated to determine if complete stenosis had occurred. In the event of incomplete stenosis, cauterization was repeated. Of the 8 dogs in the first group, 4 were cauterized and 4 used as controls. Of the 12 dogs in the second group, 8 were cauterized and 4 used as controls.

Results: Group I. Pyogenic Abscess—8 dogs: Complete stenosis was obtained in 2 dogs after cauterization had been repeated 4 and 6 times respectively. This occurred 3 and 4 months after embolization, by which time the abscess had healed with cessation of drainage through the bronchus.

Group II—Tuberculous Abscess—12 dogs: Complete stenosis accompanied by massive atelectasis occurred in 1 dog in 13 days with 1 cauterization; in 1 dog at the end of 30 days with 2 cauterizations; and in 2 dogs at the end of 2 months with 3 cauterizations. Three dogs died of complications from the abscess and in 1 dog incomplete stenosis resulted with 2 applications of the cautery. No cavities were found in the collapsed lobes at the end of 6 weeks and 8 weeks. Of the 4 controls, 2 presented cavities 1 cm. in diameter at the end of 8 weeks while the remaining 2 exhibited healing lesions $1\frac{1}{2}$ cm. in diameter at 8 and 9 weeks respectively.

Conclusions: 1. Attempted occlusion of bronchi draining a pyogenic lesion was attended by little success. 2. Pyogenic abscess cavities healed more slowly where stenosis was attempted than where it was not attempted. 3. Complete stenosis of a bronchus draining a tuberculous process was produced in 4 of 5 animals, and with fewer applications of the cautery than was necessary in pyogenic infections. 4. Tuberculous lesions healed more quickly in the collapsed lobes (without drainage), than in the inflated lobes.

5965

Rapid Healing of Embolic Lung Abscess in Atelectatic Lobes.

W. E. ADAMS. (Introduced by Edmund Andrews.)

From the Department of Surgery, University of Chicago

The relationship between massive atelectasis and pulmonary sup-puration has been a subject of much controversy during the past few years. Some believe that once massive atelectasis has developed, a suppurative process is inevitable. Others, however, are of the opinion that a suppurative lesion develops only when the obstruction producing the atelectasis contains infective organisms; or when an embolus is engrafted upon an atelectatic area.

The following experimental investigation was carried out to determine the effect of septic and aseptic emboli engrafted upon an atelectatic lung. Massive atelectasis of the left lung was produced in each of 8 dogs by the silver nitrate cauterization technique.¹ Three emboli were introduced into the jugular vein of each animal, and subsequently lodged in the lung. Two of the emboli were infected with staphylococci, spirochetes, and fusiform bacilli; the third was sterile. Each emboli contained a lead pellet and could thus be located by roentgenogram.

Results.—None of the animals died. They were sacrificed at the end of 11 days, 3½ weeks, 5 weeks, 8 weeks, and 5 months. Gross, microscopic and roentgenologic examinations were made. Over 66⅔% of the emboli lodged in the atelectatic (left) lung. Each animal received at least one septic embolus on the collapsed side. At the 11-day stage both normal and collapsed lobes presented abscesses 0.75 to 1.0 cm. in diameter. By 3½ weeks the abscesses had healed in the collapsed lobes and only a bronchial and peribronchial inflammation of a subacute or chronic nature was seen thereafter. However, cavities 1.0 cm. in diameter persisted in the inflated lobes for more than 2 months. No abscesses were produced by the bland emboli in either atelectatic or inflated pulmonary tissue.

¹ Adams, W. E., and Livingstone, H. M., *Ann. Surg.*, 1932, **95**, 106.

5966

Effect of Diet on Biliary Cholesterol.

L. E. DOSTAL AND EDMUND ANDREWS.*

From the Department of Surgery, University of Chicago.

It has been shown by us that the actual cholesterol content in bile is not as important in gall stone formation as the ratio of cholesterol to the substance which holds it in solution, namely, the bile salts. In these studies, however, we are mainly concerned with the cholesterol factor of this ratio and the effects produced on it by the administration of fats and lipoids.

Our experiments were performed on 3 dogs, using the Rous 2-way fistula. This method prevents the interruption of the enterohepatic circulation of bile and also minimizes infection of the liver. The administration, by mouth, of olive oil, butter and cream did not increase bile cholesterol. Meat, regarded by some as a precursor of cholesterol, also had no effect, throughout our series. Large amounts of bile acids were also fed by mouth and these produced no effect on either the cholesterol or bile acid content of the bile.

Further observations were made on a diabetic with a common duct tube, from which all the bile was drained for 19 days. The diabetes was mild and well controlled before the operation. For the first few days following the operation she had a slight glycosuria and some acidosis, but she rapidly returned to normal. While no blood cholesterol studies were made, one would expect somewhat of a lipemia in such a case. However, if this was present it was not reflected in an increased bile cholesterol. From the 4th postoperative day to the 12th she was receiving 130 gm. of fat per day. From the 12th to the 15th her diet was diminished to 50 gm. of fat per day. This decrease was likewise not reflected in the biliary excretion of cholesterol.

During the course of the experiment, cholesterol in doses of 1 gm. and bile acids in doses of 0.5 gm. were administered on various occasions, both separately and together. In each case they were given at 8:00 A. M. and the subsequent 4 hour specimens analyzed for 24 hours. During the entire period her stools were free from bile.

The data may be summarized as follows: The fact that the patient was a diabetic did not bring about an increase in the amount

*This work was done in part under a grant from the Douglas Smith Foundation for Medical Research.

of cholesterol either in the concentration or daily output. Variations in the dietary fats and proteins had no effect on the biliary cholesterol excretion. The feeding of cholesterol, either alone or in combination with bile acids, had no effect whatsoever. No relation existed between the cholesterol content and the volume of bile excreted. In these studies no changes in the bile salt-cholesterol ratio of sufficient magnitude to approach the critical level for precipitation have been found. The responsibility for gall stone formation seems to lie in the gall bladder rather than in the liver.

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Is Cholesterol Excreted by the Gall Bladder Mucosa?

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(Introduced by Edmund Andrews.)

From the Department of Surgery of the University of Chicago.

This problem constantly recurs in gall stone studies and the following data may throw some light on it.

Pus cells contain a very high percentage of cholesterol and for that reason exudation into the gall bladder will of course increase the cholesterol content. Rous¹ and more recently Andrews² have called attention to the great tendency of the gall bladder of the dog under experimental manipulation to become infected.

The blood of the dog contains up to 300 mg. per 100 cc. of cholesterol and the bile 10-20 mg. In fistula dogs this may even be lower. It is obvious therefore that the leakage of a single drop of blood or serum into any experimental preparation (such as a needle hole) would completely vitiate the results of most experiments.

Numerous experiments made in our laboratory, mostly for other purposes, have been reviewed and the following data secured: In 36 dogs whose common duct had been tied for 24 hours or less the cholesterol content of the bladder bile was 57 mg. per 100 cc. In 42 dogs who had had common duct ligature for 3 to 80 days there was a cholesterol content of the bladder bile of 51 mg. per 100 cc.

* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research.

¹ Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, **37**, 11.

² Andrews, E., and Hrdina, L. *Arch. Surg.*, 1931, **28**, 201.

This slight lowering may be taken as lying within the limits of experimental error. It appears, however, that in such a long series of experiments, if there were any appreciable excretion of cholesterol into the gall bladder one surely would have expected it to become evident.

In 6 human cases who had had common duct obstruction for varying periods, the cholesterol content of the bladder bile lay within normal limits.

In the cases of cystic duct obstruction reported recently by Phemister³ in which calcium carbonate gall stones occurred, the secretion of the gall bladder, although it contained large amounts of calcium, had but a trace of cholesterol in one case and none in the others.

We have tried to reproduce this condition in dogs and it has proven very difficult on account of the tendency of such gall bladders to form large abscesses. In another series the cystic duct was ligated and a fistula established. These were kept open for several weeks and drained a purulent material. Finally they became cleaner and drained clear mucus and were then allowed to close. Most of them became infected again and when reopened were mere bags of pus, but one such experiment succeeded. In this dog the gall bladder when opened contained 6 cc. of milky fluid which contained practically no pus cells. The calcium content was 580 mg. per 100 cc. and the cholesterol was but 21 mg. per 100 cc. This is in accord with the recent work on human cases.

Conclusion. The normal gall bladder mucosa secretes calcium in large amounts and cholesterol in negligible amounts.

5968

Effect of Cyanide on Respiration of the Protozoan, *Colpidium Campylum*.

ROBERT F. PITTS.* (Introduced by R. W. Gerard.)

From the Physiology Course of the Marine Biological Laboratory, Woods Hole.

The claim of Lund¹ that *Paramecium* is insensitive to KCN though questioned by Hyman² has been confirmed by Gerard and

³ Phemister, D. B., Rewbridge, A. G., and Rudisill, H., *J. Am. Med. Assn.*, 1931, **97**, 1843.

* This work was carried out under the direction of Prof. R. W. Gerard.

Hyman.³ A similar insensitivity of an alga, *Chlorella*, was described by Warburg and by Emerson,⁴ and an even more striking resistance to cyanide by a bacterium, *Sarcina lutea*, has been reported by Gerard.⁵ It seemed desirable to extend observations as to cyanide sensitivity to other protozoa. The present work is limited to the Protozoan, *Colpidium campylum*.

Methods. Pure cultures of colpidia were obtained from a single individual, and cultured on a spring water rye infusion seeded with the coccus *Achromobacter candicans* grown on agar slants for 2 days at 35° C. The colpidia were added 24 hours after the bacteria and grown for 48 hours before use. The cultures were prepared for respiration studies by gentle centrifuging, removing the bacterial layer, washing the lower sediment of protozoa and bacteria with spring water and recentrifuging, repeated 5 to 7 times. At the end the colpidia were practically bacteria free, and any of the latter still present could not have affected the respiration perceptibly. Oxygen consumption was determined by the Warburg method and the results are expressed in terms of Q_{O_2} , the cmm. of oxygen used per million animals per hour. The colpidia were enumerated with an hæmocytometer after killing with formalin. Before mounting in the manometers, the heavy protozoan suspension was diluted with a fresh phosphate buffer at pH 7.4, made by adding 5 cc. of Sorensen's phosphate to 95 cc. of spring water. The cyanide was also made fresh every day, neutralized to phenol red and dissolved in the same buffer solution. The effect of cyanide was determined by opening the manometer chamber and adding a desired amount of cyanide in the course of a run. At the relatively low pH used here, cyanide put into a side bulb volatilized into the test solution before a control run could be made. Readings were taken at 15-minute intervals for about 4 hours, and the temperature kept constant at about 24° C. R.Q.'s were determined by comparative runs with and without alkali present in the inset.

Results. Concentrations of organisms from about 100,000 to 600,000 per cc. gave the same rates of respiration, the average Q_{O_2} for 35 determinations being 151.5. These values remained approximately constant over the 4-hour period of the experiment. This

¹ Lund, E. J., *Am. J. Physiol.*, 1918, **45**, 365.

² Hyman, L. H., *Am. J. Physiol.*, 1919, **48**, 340.

³ Gerard, R. W., and Hyman, L. H., *Am. J. Physiol.*, 1931, **97**, 524.

⁴ Emerson, R., *J. Gen. Physiol.*, 1927, **10**, 469.

⁵ Gerard, R. W., *Biol. Bull.*, 1931, **60**, 227.

result is in fair accord with that of Pütter,⁶ whose values, referred to the same temperature, were about twice as high. A few determinations of R.Q. gave an average value in buffer alone of 0.65, in cyanide of 0.90. The latter may be largely due, of course, to the liberation of CO₂ by stronger fixed acids under the action of the cyanide.

Due to the manner of adding the cyanide, the first 15 minutes' respiration, after its addition, were missed. At the end of this time, in concentrations between M/1000 and M/100 the respiration was regularly depressed about 20%. With the passage of time the respiration continues to increase to values as much as 25% greater than those before the addition of cyanide. In the case of M/1000, the maximum is reached in about 2 hours, and subsequently respiration falls again to about 80% of the initial value at the end of 4½ hours. In M/500 cyanide, the rise after the initial depression is slower, occupying 3 or 4 hours, and reaches only about 110% of the original value before beginning a second decrease; and in M/100 the rise from the initial inhibition is still more prolonged, requiring over 4 hours to reach 110% of the initial value. This rise, following an initial inhibition, may be due in part to the volatilization of cyanide from the suspension fluid to the alkali inset. At the pH used, such volatilization does take place, as shown by the appearance of the cyanide effect on the organisms when NaCN buffered at pH 7.4 is put in the side arm. It is extremely doubtful, however, whether a major portion of the M/100 cyanide would have left the solution in the time involved, especially since M/1000 cyanide shows a similar behavior. Likewise the increase of respiration above normal would not be so accounted for. For *Chlorella*, Warburg found a similar stimulating effect with low cyanide concentrations. It seems probable that under the conditions of these experiments—the organisms suspended in a water solution containing a very small amount of phosphate buffer and no nutrient substance—that not more than 25% of the respiration is cyanide sensitive.

⁶ Pütter, A., *Z. f. allg. Physiol.*, 1905, **5**, 566.

5969

Phosphate in Lobster Nerve.*

VIOLET TANG, P. S. TANG AND R. W. GERARD.

From the Marine Biological Laboratory, Woods Hole.

The nerves of the large claws and walking legs of the lobster (*Homarus americanus*) with or without the abdominal ganglion chain were carefully dissected into iced mortars. They were ground and analyzed, either at once or after standing for $\frac{1}{2}$ to 3 hours in closed vessels in moist oxygen or nitrogen at 23-25° C. In a few cases companion analyses were run on nerves resting or stimulated in oxygen, stimulation being continued with fairly strong induction shocks at 100 per second for half an hour. The method of analysis was essentially that described by Fiske and Subbarow¹, involving extraction with 10% iced trichloroacetic acid, part of the filtrate being used to determine total acid-soluble phosphorus after ashing with sulphuric and nitric acids, the larger portion being neutralized and treated with calcium chloride-calcium hydroxide precipitant. After centrifuging and washing, the inorganic phosphate remained in the precipitate. Phosphagen was determined in the clear solution after reacidification with hydrochloric acid and standing at room temperature for 2 days, according to Meyerhof². Phosphate was finally estimated in each fraction by the Fiske-Subbarow method, using a total volume of 15 cc. and comparing with a phosphate standard of 8.0 mg. % strength.

The rate of hydrolysis of the phosphagen compound, as tested with acid for different time intervals, clearly showed it to be of the arginine phosphate rather than the creatin phosphate type, which is in conformity with the difference in muscle phosphagens in vertebrates and crustaceans. The usual controls of the adequacy of separation and determination demonstrated the validity of our procedure. Especially, the sum of the inorganic and phosphagen fractions separately determined was equal to the acid-labile phosphorus (Gerard and Wallen³) determined directly.

The total soluble phosphate of the nerves, at once or after a period in oxygen, averaged 41 mg. % in 7 experiments, the variation being

* Aided in part by a grant from the Rockefeller Foundation to the University of Chicago.

¹ Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1929, **81**, 629.

² Meyerhof, O., *Die chemischen Vorgänge im Muskel*. Springer, Berlin, 1930.

³ Gerard, R. W., and Wallen, J., *Am. J. Physiol.*, 1929, **89**, 108.

31 to 46. After an average stay in nitrogen of 2 hours, the value for 20 experiments was 43.4, extremes 30 to 64. The phosphagen content of those analyzed at once averaged 5.1 mg. % and showed little change on standing in oxygen. After 2 hours' anoxia, the value had fallen to 1.6. A definite decrease in phosphagen in the asphyxiated compared with the control nerve was apparent in each experiment. The phosphate present in the soluble fraction exclusive of the inorganic and phosphagen phosphate was 12 mg. %. This fraction was decreased to 8 mg. % during asphyxia. The inorganic phosphate increased in asphyxia by an amount corresponding to the decrease in the other two. The few experiments on stimulation did not lead to a definite change in the phosphate fractions, but neither the number of experiments nor the conditions of stimulation justify a conclusion on this point. Chopping and warming the nerves led to a complete breakdown of phosphagen with but little decrease in the other combined phosphate fraction. These results closely parallel previous findings on the frog sciatic³.

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Studies on Calcium. VI. Effective Dose of Calcium Gluconate Subcutaneously Administered in Completely Parathyroidectomized Dogs.*

E. BOURNS. (Introduced by A. B. Luckhardt.)

From the Physiological Laboratory, University of Chicago.

Lieberman and Szurek¹ have previously shown that parathyroid tetany can be prevented (dogs) by injecting subcutaneously large quantities of calcium gluconate (in 10% concentration). Whereas this salt of calcium is the only one so far described which can be injected subcutaneously without causing necrosis, no attempt was made to determine how *little* of a 10% solution sufficed to prevent the appearance of tetany following a single daily subcutaneous injection of this salt on the basis of cc/kg body weight in the thyroparathyroidectomized animal.

Method. Complete data were obtained from 5 dogs. The average weight of the dogs was 10 kg. (Extremes: 7.5 kg.-13.5 kg.). For the the first 2 weeks after operation the animals were put on milk and meat diet. Thereafter, on dog biscuit diet.

* Work done on grant from the Sandoz Fund.

¹ Lieberman and Szurek, *J. Pharm. and Exp. Therap.*, 1931, **41**, 333.

Syringe and needle were sterilized in 70% ethyl alcohol. The shaved skin area through which the subcutaneous injections were to be made was "sterilized" with 70% alcohol, which seemed to be a better disinfectant than an alcoholic picric acid solution. A 10% solution of calcium gluconate was used throughout.

As everyone knows who has worked with parathyroidectomized dogs, each animal presents an individual problem, probably because of the difference in the degree of parathyroid deficiency produced by the usual thyro-parathyroidectomy. The amount of calcium (in terms of cc. of a 10% solution of calcium gluconate) necessary to prevent tetany is therefore only an approximation which we hope will nevertheless be found useful to those working in this field (on dog or man).

To be certain that tetany would ensue in a given animal, no injection was given after the operation until definite signs of tetany appeared. After restoring the animal to an apparently normal status by a large dose of calcium gluconate, the dose was cut down in time until on a given dose tetany appeared.

Results and Discussion. Briefly, thyroparathyroidectomized animals will remain in normal state of health without evincing tetany if given subcutaneously 1.5 cc. of a 10% solution of calcium gluconate per kilo of body weight daily even when on a fairly high protein intake. A slight induration of the skin is likely to ensue if the solution is injected in the same area over a prolonged period of time (months).

5971

Sixteen Months Survival with Complete Biliary Obstruction in a Dog.

L. E. DOSTAL AND EDMUND ANDREWS.*

From the Department of Surgery, University of Chicago.

The longest survival that we have been able to find in jaundiced dogs is that reported by Still,¹ 205 days. For that reason the findings in one of our animals who lived over twice that long in apparent good health is of interest.

* This work was done in part under a grant from the Douglas Smith Foundation of Medical Research.

¹ Still, K. S., and Carlson, A. J., *Am. J. Physiol.*, 1929, **89**, 34.

Dog No. 454 was operated April 7, 1930, cholecystectomy and common duct divided between ligatures. The early course was normal, deep jaundice ensued and marked emaciation occurred. After several months the jaundice disappeared entirely and the dog's appetite returned, and he became sleek and glossy in appearance, ate ravenously and more than regained the weight loss. He was then put on a meat diet for 3 months, hoping to produce ascites as reported by Rowntree,² but as this did not occur he was left on the ordinary mixed diet. His stools remained entirely free from bile and extensive metabolic study was planned but had not been made except to determine that his urine was free from albumin and biliary constituents when he died very suddenly from a profuse hemorrhage from the bowel.

Autopsy showed the following significant changes: The subcutaneous, abdominal and perirenal fat were unusually large in amount and the fat was of a very light color and unusually firm in consistency. There was no connection between the biliary tract and the bowel. The common duct and the distal branches of the hepatic ducts were enormously dilated, containing a clear yellow fluid which gave negative reactions for both bile salts and cholesterol. Several small calcium-pigment stones were present in the ducts. The liver was astonishingly small, weighing but 325 gm. It was almost pure white in color with light brown streaks outlining the lobules. It was excessively friable and greasy to the touch. Sections showed that it had undergone fatty degeneration to a most astonishing degree, there being practically no liver tissue left. The stomach contained numerous small erosions and one ulcer. The duodenum had 4 large ulcers from one of which the animal had bled to death. The kidneys were nearly normal in gross appearance but the pelvis on each side contained numerous large stones as well as much fine sand, which was also found in the bladder urine. Sections of the kidneys showed practically normal glomeruli and convoluted tubules. The straight tubules contained numerous fine calcium deposits and showed slight inflammatory changes. The severe nephritis expected was conspicuously absent. The adrenals had thick cortices and an almost total absence of medulla and the lungs showed marked thickening of the alveolar walls, but no acute inflammatory changes. Bone changes were not found.

This unexplained survival of 499 days is reported to show the possibility of apparent good health and state of nutrition, with the taking over of the biliary functions by other unknown mechanisms.

² Snell, A. M., Greene, C. H., Rowntree, L. G., *Arch. Int. Med.*, 1927, **40**, 471.

5972

Effect of Prolonged Stasis on the Bile Salt-Cholesterol Ratio.

M. GOFF, L. HRDINA AND EDMUND ANDREWS.

From the Department of Surgery, University of Chicago.

In our studies of the relative concentration of cholesterol in the bile and bile salts which hold it in solution, the element of time in which the bile lies in the gall bladder is important. If prolonged stasis produces any differential absorption it would profoundly influence the likelihood of cholesterol precipitation. As shown in previous papers^{1, 2} the ligature of the cystic duct in a dog brings about marked infection in the gall bladder but common duct ligature, although it usually results in the presence of bacteria in the bile, only occasionally causes a marked cholecystitis. In the series of experiments reported below pathological studies of the gall bladders are reserved for a later paper and only the chemical investigations are included here.

Fifty-one dogs were used and the common ducts ligated and the animals sacrificed or died at various periods.

TABLE I.

Time days	No. of dogs	Chol.	B.S.	B.S./Chol. ratio
1	15	62	3440	55
3-5	9	69	3157	45
6-12	12	78	4741	61
13-42	8	24	1219	52
44-80	7	41	2034	49

It can be seen from these data that there is no differential absorption of either bile salts or cholesterol. Prolonged stasis does not cause any change in the bile salt-cholesterol ratio, and hence cannot be considered as a factor in gall stone formation *per se*. Other points to be noted are the wide fluctuations in both the bile salts and cholesterol but the tendency of the ratio between the 2 to remain at a fairly definite level.

Of 51 operated dogs 25 died and 26 were killed. In the 2 series the average cholesterol content was the same but in the dogs who died the average bile salt content was 25% lower. This observation is of importance as it throws considerable doubt on chemical examination of post mortem material whether human or experimental in

¹ Andrews, E., Hrdina, L., *Am. J. Med. Sci.*, 1931, **181**, 478.

² Andrews, E., Hrdina, L., *Arch. Surg.*, 1931, **23**, 201.

studies of this sort. Probably the liver during the agonal period secretes a bile with considerably less bile salt content. Whipple³ showed that the diseased liver behaved in this manner. These observations were confirmed⁴ in our laboratory and it was also shown that the liver if diseased might secrete uncombined bile salts which of course do not show in the gasometric analysis of Van Slyke.

³ Whipple, G. H., *Phys. Rev.*, 1922, **2**, 440.

⁴ Andrews, E., Hrdina, L., Dostal, L. E., *Arch. Surg.*, to be published.

Minnesota Section.

University of Minnesota, January 20, 1932.

5973

Lactose Fermenting Anaerobes in Soil and Their Relation to Sanitary Water Analysis.

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The suggestion has been made that the search for lactose fermenting anaerobes be made routinely in place of, or in addition to, the lactose fermenting aerobes (*Bacterium coli*). The purpose of this work was to determine the advisability of such a procedure. If lactose fermenting anaerobes are to serve as an index of fecal pollution in water, it must be shown that they are constantly present in feces and that they are not found in large numbers in soil or elsewhere in nature where they might get into water supplies. Furthermore, as stated by Levine¹, it ought to be shown that they are able to exist in water for a short time (but only for a short time) after the water has been freed from pathogens. It would seem requisite also that they be readily susceptible to detection without too much time, equipment, or effort.

There is no doubt that lactose fermenting anaerobes, including *Clostridium welchii*, are normally present in human feces, but not all authors have found them in all samples studied, possibly because of the technical difficulties of the isolation of anaerobes.

As to their incidence in nature outside of the intestinal tract, opinions differ. Greer², pointing out the importance of lactose fermenting anaerobes, ignores the possibility of their wide distribution in soil. Topley and Wilson³ believe that the normal habitat of anaerobes is soil, and that their presence in sewage and feces is inci-

¹ Levine, M., *Iowa State Col. Eng. Exp. Sta. Bul.*, 62, 1921.

² Greer, F. E., *J. Infec. Dis.*, 1928, 42, 501.

³ Topley, W. W. C., and Wilson, G. S., *Principles of Bacteriology and Immunology*. Wm. Wood & Co. 1929.

dental. This opinion, while possibly extreme, is, in the light of the literature and the work to be reported, probably nearer the correct one than that of Greer, and others, namely, that *Cl. welchii* is to some extent an index of fecal pollution.

Experimental. It is obvious to anyone who has isolated anaerobes that it is impractical to use *Cl. welchii* as an index of fecal pollution if it is necessary to isolate the organism each time a sample of water is to be tested. While not particularly involved, the isolation of anaerobes takes far too much time and is too uncertain for routine water work. As a substitute for isolation there are a few more or less satisfactory criteria used to identify this organism and its close relatives. First, the stormy fermentation of milk may be used. The authors used the following method: Dilutions of soil were added to milk, and heated for 10 minutes at 75° to drive off excess oxygen and to kill the vegetative cells including *Bacterium aerogenes* and *B. coli*. Sterile paraffin wax was added, as in the Weinzirl⁴ technique. Gassy fermentation results in lifting the paraffin plug and the stormy fermentation may be detected by observing the character of the clot. It should be stated that all gradations of stormy fermentation from the extreme to none at all were observed, together with gas formation, sufficient to loosen the paraffin plug, and an arbitrary line of demarcation had to be drawn. If only lactose fermentation is desired, lactose broth with paraffin wax plugs or lactose broth with gas traps is applicable. To determine the approximate numbers of spores in soil, the dilution method was used. Five 1 cc. replicate samples of decimal dilutions were used, and the numbers determined from the tables of Halvorson⁵.

The method of Wilson and McBlair⁶ may also be used. This is not specific for *Cl. welchii*, or even for anaerobes, but Wilson and McBlair, Adams,⁷ and Greer² have suggested its use. For our work we used a modification of this method since large petri plates were not available. Inverted standard size petri plate bottoms were inserted in tops and sterilized. The dilutions were placed in the depression of the top, and the iron, sulphite, and previously boiled dextrose agar, poured and mixed with the dilution. The bottom was placed over it, the convex side of the bottom fitting into the concave side of the top, care being taken to eliminate all air bubbles. Sterile agar was placed around the circular border formed by the

⁴ Weinzirl, J., *Am. J. Pub. Health*, 1921, **11**, 149.

⁵ Halvorson, H. O., unpublished data.

⁶ Wilson, W. J., and McBlair, E. M. McV., *J. Path. and Bact.*, 1924, **27**, 119.

⁷ Adams, B. A., *Water and Water Eng.*, 1929, **31**, 412.

insertion of the inverted bottom in the top, thus making anaërobiosis more complete. Tests with pure cultures of *Clostridium botulinum* and *Cl. welchii* have demonstrated that anaerobes grow well in such plates. All plates were incubated in moist chambers to prevent cracking of the agar and subsequent aerobic conditions near the cracks. The colonies of five or ten replicate plates were counted, only those being considered which showed the typical black colonies which the British authors found to be usually *Cl. welchii* and its relatives.

By means of these three methods, determinations of the "numbers" of *Cl. welchii* in soil were obtained. The soil samples were from forests, peat bogs, or unmanured fields. Most of them were virgin soils, that is, they had never been cultivated although they may have been logged off. Fecal pollution, if present, must have been at a minimum. Four of the samples had been air dried for five years in the laboratory. The samples plated on Wilson and McBlair's medium were not the same as those inoculated into milk. Although *Cl. welchii* has been found in soil by many investigators, it is believed that these are the only quantitative figures in the literature.

Seven separate strains of lactose fermenting anaerobes were isolated from the soil. They fermented glucose, lactose, sucrose, and maltose, forming acid and copious gas. They caused a stormy fermentation of milk, and liquified gelatin. They were either *Cl. welchii* or closely related anaerobes. Morphologically, they were similar to a strain of *Cl. welchii* obtained originally from Hall's laboratory.

The results show that if lactose fermentation with gas or a stormy fermentation of milk inoculated with a heated suspension or a blackening with typical large colonies on Wilson and McBlair's⁶ medium be taken as a criterion for *Cl. welchii*, these organisms can scarcely be taken to indicate fecal pollution. It is hardly conceivable that organisms so numerous in soil would fail to get into water supplies. A sudden increase in their numbers may indicate a surface drainage due to rain or to agitation of the mud in streams, or to many other factors but not necessarily to sewage pollution. The spores are known to be resistant, and there is no reason to believe that they will not exist in water long after the water has been freed from pathogens by treatment or autopurification. For these reasons, it would seem that sufficient data have accumulated to indicate that lactose fermenting spore formers are so numerous in soil as to preclude their use for indicating fecal pollution. And if, as some maintain, these organisms in themselves are dangerous when taken

TABLE I.

Number of lactose fermenting spores in soil determined by stormy fermentation of milk and by gas formation, using dilution method.

Soil Samples	No. of tubes showing stormy fermentation. (5 inoculated with 1 cc. soil dilution)			Most probable No. per gm. of soil	No. of tubes showing gassy fermentation. (5 inoculated with 1 cc. soil dilution)			Most probable No. per gm. of soil
	1:10	1:100	1:1000		1:10	1:100	1:1000	
1	5	1	0	33	5	5	4	1620
2	5	4	1	171	5	5	2	542
3	5	2	0	49	5	4	1	171
4	5	5	4	1620	5	5	5	>10,000
5	5	5	3	918	5	5	5	>10,000
6	5	5	2	542	5	5	4	1620
7	1	0	0	2	4	0	0	13
8	5	5	5	>10,000	5	5	5	>10,000
9	5	4	2	221	5	5	5	>10,000
10	4	2	1	27	5	2	1	60
11	5	0	0	23	5	1	0	33
12	5	5	3	918	5	5	3	918
13	3	0	0	8	3	0	0	8
14	5	3	1	109	5	5	3	918
15	4	1	0	17	5	3	0	79
16	4	0	0	13	5	1	0	33
17	5	5	3	918	5	5	5	>10,000
18	5	5	1	349	5	5	5	>10,000
19	5	5	4	1620	5	5	5	>10,000
20	5	4	1	171	5	5	3	918

TABLE II.

Numbers of bacteria causing large black colonies on Wilson and McBlair's anaerobic medium.

Sample	No. per gm. of soil	Sample	No. per gm. of soil
1	532	10	25
2	483	11	160
3	860	12	42
4	1000	13	390
5	3700	14	5600
6	230	15	11
7	6	16	130
8	320	17	9
9	6210	18	4900

into the alimentary tract, such uncooked foods as carrots, tomatoes, radishes, lettuce, strawberries, cucumbers, and others grown in, on, or near the soil, should be viewed with suspicion. Likewise, most water supplies, if the above be true, are potential sources of *Cl. welchii* infection, since chlorination does not eliminate all the spores. However, evidence that these organisms from the soil are dangerous in food or water is far from conclusive.

5974

A Study of Bovine Blood, Urine and Feces for the Presence of
Bact. Abortus Bang.*

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Shaw¹ was able to demonstrate the presence of *Micrococcus melitensis* in the blood stream of patients suffering from undulant fever. Horrocks² demonstrated the organism in the urine but was not able to find it in the feces of these patients. Amoss and Poston^{3, 4} reported a technic by which they were able to isolate, using cultural methods, Brucella organisms from the feces of undulant fever patients. There has been little reported on the presence of *Bact. abortus Bang* in the blood, urine, and feces of cattle affected with Bang's disease. Zeller⁵ attempted to infect six cows by subcutaneous injections of *Bact. abortus Bang*. Two of these were inoculated with suspensions heated at 57° C. for 10 minutes. He studied the blood, urine and feces and was unable on autopsy to find the organism in any tissue of the body. Long⁶ studied the urine of cattle affected with Bang's disease. Studies were made on nineteen different animals over a period of ten months. Cultural methods gave negative results from 109 samples. Friess⁷ examined 131 bovine blood samples by microscopic, cultural, and animal inoculation methods with negative results. Soule⁸ made blood cultures of a large number of dairy cows. Two series of tests were made; one series resulted in 299 positive blood cultures from 2,237 animals in which agglutinins were present in the blood. The second series gave 206 positive blood cultures from 2,607 agglutinin positive animals. Forty animals gave positive blood cultures without concomitant blood agglutinin.

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¹ Shaw, E. A., Reports of the Commission Appointed to Investigate Mediterranean Fever. 1905-7, Parts I-VII, 19.

² Horrocks, R. A., Reports of the Commission Appointed to Investigate Mediterranean Fever. 1905-07, Parts I-VII, 19, 22, and 36.

³ Amoss, Harold L., and Poston, Mary A., *J. Am. Med. Assn.*, 1929, **93**, 170.

⁴ Amoss, Harold L., and Poston, Mary A., *J. Am. Med. Assn.*, 1930, **95**, 482.

⁵ Zeller, H., *Arch. f. wissenschaft. u. prakt. Tierh.*, 1922, **49**, 65.

⁶ Long, L. E., Thesis, Michigan State College, East Lansing Mich. 1927.

⁷ Friess, Ferdinand, *Jahr. Vet.-Med. Neumann-Kleinpaul u. Zietzschmann*, 1931, **50**, 1122.

⁸ Soule, M. H., *Ier Congres International de Microbiologie*, Paris, 1930, **1**, 606.

We have studied the presence of *Bact. abortus* Bang in the blood, urine and feces of cattle. Samples of blood, urine and feces were collected in the following manner: Each animal was bled in a standing position. Blood was obtained by vena puncture of the jugular and collected in 100 ml. centrifuge tubes containing 1 ml. of a 20% solution of sodium citrate. Samples of urine were collected by catheterization whenever possible. In order to prevent possible contamination the external genitals and vaginal canal were washed with clean water. When this procedure caused evacuation of the bladder before the catheter could be introduced the first ounce or 2 of urine was allowed to escape and the required amount was then collected in sterile flasks. Feces were collected by dilating the anus which usually brought about evacuation of the posterior bowel. The samples were placed in small petri dishes.

Fifteen to 20 gm. of feces were suspended in 250 ml. of saline solution and allowed to stand over night. In the morning the suspension was filtered through 4 layers of hospital gauze to remove the coarser particles. The filtrate was then placed in two 100 ml. centrifuge tubes and centrifuged at low speed, 400-500 r.p.m., for 5 minutes. This was intended to throw the larger particles to the bottom of the tube. The supernatant fluid was poured into 2 other 100 ml. centrifuge tubes together with 1 ml. positive *Bact. abortus* serum with an agglutination titre of more than 1-1000 dilution. These tubes were then placed in a water bath at 37°C. for two hours in order to clump *Bact. abortus* if present. The tubes were then removed from the water bath and centrifuged at 1000 r.p.m. for 10 minutes. The supernatant liquid was discarded and the sediment washed by resuspending in 100 ml. of saline solution. This was repeated twice. Some of the sediment was streaked on gentian violet plates and incubated under 10% CO₂. The rest of the sediment was resuspended in 10-15 ml. of saline solution and 2 guinea pigs were inoculated with 2 ml. amounts.

Urine samples were treated in the same manner except that they were used immediately and were not filtered through gauze. Five ml. amounts of the final sediment after washing were injected intraperitoneally into guinea pigs.

The samples of citrated blood were collected in each of two 100 ml. centrifuge tubes. These were centrifuged at high speed (2000 r.p.m.) until the serum was free of blood cells. The serum was then pipetted off; and the cells suspended in saline solution. These were centrifuged again and the above process repeated twice. The 2 samples of blood cells were then mixed together and cultured by smear-

ing on serum agar and gentian violet plates. Guinea pigs were inoculated with the washed cells; 6-10 ml. amounts were injected intraperitoneally into each of 2 pigs.

These inoculation technics were tested by putting small numbers of *Bact. abortus* Bang into samples of blood, urine and feces and treating them as above. *Bact. abortus* Bang was recovered in every instance by guinea pig inoculation.

Cultural examinations of the blood cells, urine and feces were negative for the Bang organism. Gentian violet plates did not inhibit the colon bacilli. There was always a heavy growth of these organisms on the plates of cultures from urine and feces. Eosin methylene blue plates as recommended by Amoss and Poston (loc. cit.) were of little value as difficulty was experienced in getting *Bact. abortus* Bang to grow on them.

Fifteen cows positive to the test tube agglutination blood test for *Bact. abortus* Bang were obtained for study immediately after calving or aborting. Bacteriological examinations were made of the following materials: colostrum, milk, fetal membranes, feti, blood, urine and feces. Studies were made of blood, urine, and feces in some cases as long as 47 days after the termination of pregnancy.

Attempts were made to infect artificially 4 animals by drenching (1) with saline suspensions from cultures of *Bact. abortus*, (2) stomach contents of *Bact. abortus* infected feti and by dropping saline suspensions of *Bact. abortus* into the conjunctival sac. *Bact. abortus* Bang was isolated at the time of calving or aborting from 2 of these showing that they became positively infected. Samples of blood, urine and feces from these 4 animals were studied both before and after calving or aborting.

One hundred and twenty-three samples of citrated blood were examined, 119 were negative and 4 positive; 119 urine samples, 102 negative, 17 positive; 118 feces samples, 116 negative and 2 positive.

These results indicate that *Bact. abortus* Bang is not present in large numbers in the blood stream of infected cows. It will be noted that *Bact. abortus* Bang was isolated 4 times from the blood out of 123 samples and 2 of these positive results were from a single animal. The longest period of time after calving or aborting that *Bact. abortus* Bang was isolated from urine was 12 days. This is within the period of time that *Bact. abortus* Bang has been isolated from vaginal discharges.⁹ It is evident that it would be

⁹ Fitch, C. P., Delez, A. L., and Boyd, W. L., *J. Am. Vet. Med. Assn.*, 1930, 29, 680.

impossible to be absolutely certain that the organisms did not get into the urine through its contact with the vaginal and uterine discharges. This indicates that the urine may be a means of dissemination of *Bact. abortus* Bang. Two positive feces inoculations were obtained from 2 different animals. The samples were collected one and 3 days after aborting. This small number of positive results would lead one to believe that there is not an active infection in the intestinal tract but that the presence of the organism in the feces is probably due to eating materials contaminated with *Bact. abortus* Bang.

Blood cultures were prepared from blood taken from the jugular vein. The skin and hair coat of the head, neck, and withers was thoroughly moistened. An area 15x10 cm. on either side of the neck usually midway between head and shoulders was shaved and painted with tincture of iodine. An incision was made through the skin and the incised area was lightly touched with iodine. A sterile 30 ml. all glass syringe with a 16 gauge hypodermic needle attached was then used in aspirating the blood. The blood was transferred to flasks containing 250 ml. of 10% serum beef infusion broth. These flasks were incubated at $37\frac{1}{2}^{\circ}\text{C}$. under 10% CO_2 for 10 days. Subcultures were made at intervals on gentian violet plates and serum agar slants. The results obtained from these blood cultures were very unsatisfactory. Sixty cultures were made in all. Four of these cultures were sterile. The other 56 cultures showed gross contaminants. From 31 of these the gentian violet plates remained sterile whereas the serum agar slants showed a variety of growths. *Bact. abortus* Bang was not found in a single instance from blood cultures. In the case of one blood sample a small number of *Bact. abortus* Bang organisms were added. *Bact. abortus* was recovered when subcultured on gentian violet plates. This broth culture was contaminated with gram positive organisms but these did not grow on gentian violet plates.

Summary. *Bact. abortus* Bang was not found often in the blood, urine and feces of cattle known to be infected with this organism. Guinea pig inoculation proved to be a much more satisfactory method of isolation than cultural procedures.

Effect of Intravenous Injections of Peritoneal Fluids Recovered from Dogs Dying of Experimental Intestinal Strangulations.*

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From the Department of Surgery, University of Minnesota.

The lethal factors in strangulation obstruction are believed by most workers to be due to the absorption of toxins which are elaborated within the lumen of the strangulated loop or within its wall. If this be the case, any toxemia that may result must be secondary to the absorption of these toxins into the general system. This implies either absorption through the mesenteric lymphatics draining the loop, the mesenteric veins or else transperitoneally. However, there is little evidence that in a strangulation of any magnitude the veins or lymphatics from the strangulated loop are still capable of absorbing fluids or other products. The most recent investigators concede that if there is any absorption of toxic products it must be transperitoneally.^{1, 2}

Methods. We therefore undertook to investigate the toxicity of the peritoneal fluids recovered from the abdomens of dogs dying from various types of intestinal strangulations. In order to be certain that these fluids reached the general circulation they were introduced directly into the leg veins of normal dogs. At the time the fluids were introduced, a carotid blood pressure tracing was being made so that the immediate effect on the pressure might be accurately observed. Amounts varying between 20 and 185 cc. were used. Fourteen experiments were carried out on 13 different dogs. There were no immediate or delayed effects in 10 of the animals. In these 10 instances the peritoneal fluid had been obtained from animals that had died without gross rupture of the strangulated segment. In the 4 remaining experiments there was an immediate effect which consisted of a marked sudden fall in blood pressure followed by a sharp rise and the next day all 4 animals were dead. In 3 of these 4 instances the peritoneal fluid was obtained from animals in which the loops had ruptured. The fourth animal had died with an intact loop, but the wall of the gut was so necrotic that it

* This work was supported in part by Grant 244 allowed by the Committee on Scientific Research of the American Medical Association.

¹ Stone, H. B., and Firor, W. M., *Trans. South. Surg. and Gynec. Assn.*, 1924, **37**, 173.

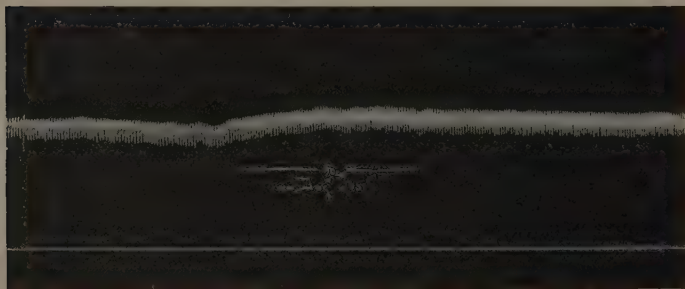
² Schönbauer, L., *Arch. f. klin. Chir.*, 1924, **130**, 427.

had to be handled with extreme care lest it rupture at any moment. The peritoneal fluid from these 4 dogs gave off a foul odor. Bacteriological examinations revealed the presence of innumerable an-

TABLE I.

Strangulation Dogs			Normal Dogs	
Exp.	Duration hrs.	Condition of bowel	Amount injected cc.	Result
33*	4	Intact	100	No immediate or delayed reaction. 2 dogs.
34*	5½	"	50	No immediate or delayed reaction.
36*	6	"	50+	No immediate or delayed reaction other than slight (10 mm.) rise and fall in blood pressure to normal.
			90	
			blood serum	
41*	8½	"	185	No effect on blood pressure other than slight rise—no delayed effect.
37*	6½	"	80+45	No immediate or delayed effect other than slight rise in blood pressure.
42*	19	"	120	No immediate or delayed effect other than slight rise in blood pressure.
43*	18	"	90	No immediate or delayed effect other than slight rise in blood pressure.
44*	17	Ruptured	100	Abrupt fall in blood pressure followed by rise—dead next morning.
50†	1	Intact	20	No effect.
47†	7	"	20	No effect.
45†	18	Intact, very necrotic	20	Abrupt fall in blood pressure to 60 mm. followed by abrupt rise to 135 mm. Dog killed.
48‡	15	Ruptured	40	Very abrupt fall in blood pressure to 85 mm. followed by a rapid rise to 240 mm. Dead next day.
46‡	27	"	20	Marked fall in blood pressure followed by gradual rise. Dead next day.

*Encirclement. †Venous. ‡Complete.



TRACING I.

Effect on blood pressure of intravenous injection of peritoneal fluids obtained from a dog dying of intestinal strangulation without rupture of loop.



TRACING 2.

Effect on blood pressure of intravenous injection of peritoneal fluid obtained from a dog dying of intestinal strangulation with a ruptured loop.

aerobic and aerobic organisms. For details of experiments see the table and tracings.

Conclusions. The peritoneal fluid obtained from dogs dying of strangulation obstruction is non-toxic, when injected intravenously into normal dogs providing the strangulated loop is not ruptured or about to rupture and the fluid does not give off a foul odor. We do not deny that the peritoneal fluid may contain toxic products, but they are not demonstrable, at least on injection into other dogs until rupture of the loop.

5976

Can Immunity to Tetanus be Produced by the Oral Route?

MELVILLE H. MANSON. (Introduced by Owen H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

The problem of producing an acquired, systemic immunity to diseases of bacterial origin by the oral administration of organisms or their growth products has been studied sporadically since 1880. The majority of investigations have been undertaken in diseases that have either the portal of entry or their pathology in the gastrointestinal tract, as in typhoid fever, dysentery, and intestinal anthrax. This study was undertaken because of the undisputed opinion based on experimental evidence in the literature that a systemic immunity to tetanus could be produced by the oral administration of tetanus bacilli.

Ten Broeck and Bauer¹ observed that tetanus was less frequent among the Chinese people than those of the United States, reported the finding of tetanus bacilli in 34.7% of the stools of 78 Chinese. They later reported the examination of the blood sera of 26 individuals known to have tetanus bacilli in their digestive tracts, all of which had "appreciable amounts of antitoxin".² The blood sera of 30 persons in whom no tetanus bacilli were found in the stools contained no antitoxin, with 2 exceptions. In 40 specimens of serum from persons not examined for bacilli, they report the finding of antitoxin in 37.5%, which corresponded quite well to the previously reported 34.7% positive stools. In 1923, Ten Broeck and Bauer³ reported that they were able to produce an active immunity in guinea pigs by the oral administration of tetanus bacilli. As a preliminary procedure in their animal experiments they cultured the stools of a number of their stock guinea pigs and found that 27% had tetanus spores or bacilli in the intestinal tract and also antitoxin in the blood serum. They report the successful production of antitoxin in the blood to such an extent that 0.5 cc. protected against 10, 25, and 75 minimum lethal doses of toxin, appearing in approximately one month after the establishment of carriers.

Experiments. Two series of guinea pigs were used in the first set of experiments. Series I, 8 animals were fed tetanus bacilli each day. Stool cultures were made at intervals, the filtrate of which produced tetanus when injected into white mice, thus establishing that the animals had the organisms in their digestive tracts. Series II, 8 animals, were fed toxin each day, by sucking the fluid from a small pipette. Ten mg. of toxin was mixed with 10 cc. of saline, each animal receiving 1 cc. each morning. Before commencing the feedings the stools of all the animals were cultured in glucose brain broth. If, on microscopic examination, there appeared any organisms which were morphologically similar to the tetanus bacillus, the culture was filtered through a Berkefeld filter and the filtrate injected into a white mouse. None of the filtrates produced any symptoms of tetanus. Tests for immunity in the form of antitoxin in the blood serum were made either by directly injecting one m.l.d. of toxin (1/50 mg.) or more intramuscularly or by withdrawing heart's blood, pipetting off the serum and mixing this with tetanus toxin. The serum and toxin was allowed to stand at room temperature for one hour before injection. Fresh solutions were made each

¹ Ten Broeck, C., and Bauer, J., *J. Exp. Med.*, 1922, **36**, 261.

² Ten Broeck, C., and Bauer, J., *J. Exp. Med.*, 1923, **37**, 479.

³ Ten Broeck, C., and Bauer, J., *Proc. Soc. Exp. Biol. and Med.*, 1923, **21**, 267.

day from dried toxin, both for the daily feedings and for injection purposes.

PROTOCOL SUMMARY SERIES I.
Fed tetanus bacilli. Feedings started on February 14, 1931.

Pig	Wgt. gm.	Injected with	Time days	Dead in days	Control Dead in days
1	400	1 m.l.d.	37	3	6
2	300	1 m.l.d.	37	6	8
3	330	1 m.l.d.	40	4	9
4	370	Animal died 21st day after feedings were started. No tetanus.			
5	440	1 m.l.d.	40	6	8
6	300	1 cc. serum plus 1 m.l.d. into control pig	40	6	8
7	350	Animal died 18th day. No tetanus. No injection.			
8	380	1 cc. serum plus 1 m.l.d. into control pig	96	8	7

Pigs Nos. 6 and 8 from whom serum was taken were also injected with 1 m.l.d. of toxin. Died in 5 and 7 days respectively. Controls died in 4 and 10 days.

In Series I there were 6 intramuscular injections of 1 m.l.d. of tetanus toxin given to animals which had been fed tetanus bacilli from 37 to 96 days. None of the animals showed any evidence of resistance to the disease. Peculiarly, the majority died at an earlier date than the control animals. In 2 instances serum from the animal which had received bacilli by mouth when mixed with 1 m.l.d. of toxin failed to protect another animal.

SERIES II.
Fed tetanus toxin. Feedings started on January 23, 1931.

Pig	Injected with	Time days	Result	Control
1	1 m.l.d.	22	Dead 13 days	Dead 8 days
2	1 m.l.d.	35	Developed tetanus	
3	1 m.l.d.	35	Alive 62 days Developed tetanus Recovered	
			On 108th day, 1 cc. serum plus 1 m.l.d. toxin into control resulted in death 8th day	
4	1 m.l.d.	59	Dead 9 days	Dead 6 days
5	1 m.l.d.	59	Dead 6 days	Dead 7 days
6	Died 35th day. No tetanus. No injection.			
7	2 m.l.d.	65	Dead 3 days	No control injected
8	Died 22nd day. No tetanus. No injection.			

In Series II the results of 6 intramuscular injections of 1 m.l.d. of toxin given to animals who had received 1 mg. of toxin by mouth for periods from 22 to 65 days were lethal to each of the pigs within a time comparable to the control animals except for Nos. 2 and 3. Pig No. 2 was unfortunately used for another experi-

ment and the opportunity to test his serum for the presence of anti-toxin was lost. The blood serum of No. 3 failed to neutralize 1 m.l.d. of toxin after 108 days of oral administration of toxin.

Because the experiments were so few in number and because the results were at such variance with those previously reported by others, the third series of experiments was made.

SERIES III.				
Fed 1 mg. or more of toxin each day. Feeding started on Sept. 14, 1931.				
Fig	Injected with	Time days	Result	Control
1	1 m.l.d.	55	Dead 13 days	
2	1 m.l.d.	55	Developed tetanus. Lived	
	2 m.l.d.	89	Dead 6 days	
3	1 m.l.d.	55	Developed tetanus. Lived	
	2 m.l.d.	89	Dead 6 days	
4	1 m.l.d.	90	Dead 5 days	2 control animals injected
5	1 m.l.d.	90	Dead 7 days	1 m.l.d. toxin.
6	2 m.l.d.	90	Dead 4 days	Died 6 days.
7	2 m.l.d.	90	Dead 6 days	2 control animals injected
8	2 m.l.d.	90	Dead 5 days	1 m.l.d. toxin.
9	2 m.l.d.	90	Dead 3 days	Died 6 days.
10	Pig developed an ulceration on side of face; a week after this was noticed the animal developed tetanus (October 16—33 days of toxin), from which it died in 9 days.			
11	1 cc. serum from No. 5 plus 1 m.l.d. toxin	55	Dead 9 days	
12	1 cc. serum from No. 7 plus 1 m.l.d. toxin	55	Developed tetanus. Lived.	

Pig No. 10 completed an experiment without having any injection of toxin. It was thought that the portal of entry was through the ulcer, contact being obtained from spillage at the time of its daily feedings. With the exception of one animal, 1 or 2 m.l.d.'s of toxin was lethal in every instance.

Comment. Eighteen intramuscular injections of tetanus toxin were made into guinea pigs which had received the same toxin orally for periods of from 22 to 108 days. There was no evidence that they had established any immunity to the disease. Six animals which had been fed tetanus bacilli for 96 days failed to show any immunity to tetanus either when injected with tetanus toxin or when their blood serum was mixed with toxin and injected into a normal guinea pig.

Conclusions. 1. Cultures of 16 stools from stock guinea pigs showed no tetanus bacilli. 2. Guinea pigs fed with tetanus bacilli do not develop an active immunity to tetanus. 3. Guinea pigs fed with tetanus toxin are as susceptible to tetanus as normal animals.

5977

Effect of Viscosity on Specific Agglutination.

HOBART A. REIMANN.

From the Department of Medicine, University Hospital, University of Minnesota.

In a previous note¹ reference was made to the enhancing effect of increased viscosity on the agglutinative property of specific immune serum. Plasma of abnormally increased viscosity caused agglutination of bacteria to take place in higher dilutions of specific immune serum than normal plasma. Recently, opportunity was afforded to repeat the experiments using the plasma obtained from a patient with hyperinosis. The patient's plasma contained 10.12% protein, of which 5.48% were fibrinogen, 2.27% euglobulin, 1.47% pseudoglobulin I and II and 0.90% albumin (Dr. Grace Medes). The viscosity of this plasma tested with the Hess viscosimeter was over 7, as compared with normal plasma which, according to the technic employed, varied between 1.6 and 1.8.

Tests were made by arranging parallel series of tubes, one row containing plasma with a viscosity of 7, the other with normal plasma with a viscosity of 1.7, from a normal individual. To each row was added type II antipneumococcus serum (New York State Department of Health) so that dilutions ascended from 1-10 to 1-160 as shown in Table I. A suitable amount of type B Friedländer bacilli (kindly supplied by Dr. L. A. Julianelle) which agglutinate specifically in type II antipneumococcus serum was added to each tube. The tubes were incubated for 10 minutes at 37°C., during which time they were inverted gently at frequent intervals to insure thorough mixing. At the end of this time, smears were made from each tube and stained.

Results. There was no agglutination demonstrable in the normal plasma in any tube. Marked clumping was present in most of the tubes containing plasma of high viscosity. Clumping diminished as

TABLE I.

Dilution of type II serum	1-10	1-20	1-40	1-80	1-160	No serum
Normal plasma	—	—	—	—	—	—
High viscosity plasma	+++	+++	++	++	+	—

Effect of high viscosity plasma, contrasted with normal plasma on specific agglutination of type B Friedländer bacilli in type II antipneumococcus serum dilutions. Microscopic agglutination after 10 minutes at 37°C.

¹ Reimann, H. A., *Science*, 1931, **74**, 513.

the dilution of specific immune serum increased as shown in Table I. Clumping did not occur in the tube containing no immune serum.

A similar phenomenon can be demonstrated by increasing the viscosity of normal plasma with gum acacia. The following experiment illustrates the effect of various dilutions of acacia, while the concentration of specific immune serum in plasma remains constant.

TABLE II.

Dilution of acacia gum	10%	5	2½	1½	Control
Normal plasma containing type II antipneumococcus serum 1-10	++++ (Disc)	+++	+	±	—

Effect of acacia in enhancing the specific agglutinative effect of type II antipneumococcus serum on type B Friedländer bacilli. Macroscopic agglutination after 2 hours at 37°C.

Clumping is markedly increased in the stronger concentrations of acacia. Agar, gelatin, glycerin, egg white, and starch paste are less effective than acacia in favoring agglutination.

5978

Streptococcic Agglutination in Glomerulonephritis.

B. J. CLAWSON AND MACNIDER WETHERBY. (Introduced by George Fahr.)

From the Departments of Pathology and Medicine, University of Minnesota.

The streptococcic agglutinating titers of the serums of 20 patients with glomerulonephritis were determined for a strain of streptococci recovered from the blood of a patient having acute rheumatic fever. This strain had been kept on media for 9 years. It produced a green discoloration faintly on sheep blood agar when incubated at 37°C. for 24 hours. It cross agglutinated in high dilution (1:50,000 and more) with many other strains isolated from patients with acute rheumatic fever and with several strains recovered from the blood of patients having chronic arthritis.

In determining the titers the various dilutions of the serums and the suspensions of the streptococcus were incubated in Wassermann tubes in a water bath at 40°C. for 2 hours and then placed in the icebox until the next morning when the agglutinating titers were read.

All serums agglutinated in dilutions as high as 1:800. Five

agglutinated at 1:800, ten at 1:1,600, one at 1:3,200, three at 1:6,400, and one at 1:12,800. The greatest number in any one dilution agglutinated at 1:1,600.

The height of the agglutinating titers of the serums in glomerulonephritis with the above strain of streptococcus was decidedly higher than that found with the same strain with the serums of 110 normal individuals, 50 patients with acute rheumatic fever, and 300 patients with chronic arthritis. The greatest percentage of the serums in these 3 conditions agglutinated the streptococcus at the dilution of 1:200, but in each there were many serums which failed to show agglutinins in dilutions as low as 1:50 and 1:100.

The height at which agglutination with the serums in glomerulonephritis took place was also higher than that found in a limited number of cases of scarlet fever, bacterial endocarditis, and ulcerative colitis.

Several of the serums from the cases of glomerulonephritis were tested for agglutinins for *B. typhosus*. A positive agglutination occurred in one at a dilution of 1:50.

Three serums from patients suffering from renal insufficiency associated with essential hypertension were also tested for streptococcic agglutinins. The highest titers at which agglutination occurred were 1:50 in 2 and 1:400 in one.

The relative height of the streptococcic agglutinating titers in glomerulonephritis suggested (1) an etiologic relationship and (2) a possible biologic method for differentiating renal insufficiency due to glomerulonephritis from renal insufficiency associated with primary hypertension.

5979

Subcutaneous Nodules in Chronic Arthritis.

B. J. CLAWSON AND MACNIDER WETHERBY. (Introduced by George Fahr.)

From the Departments of Pathology and Medicine, University of Minnesota.

In 200 patients with chronic arthritis subcutaneous nodules were found in 59 (29.5%). Forty-nine of the 58 cases were 50 years of age or older.

The nodules when removed often had a definite capsule but in some cases this was poorly defined. When sectioned and examined grossly, multiple areas of necrosis were usually seen, surrounded

by fibrous tissue. Necrotic and mucinous material could frequently be expressed from the center.

The nodules from 20 cases were examined microscopically. They were found to be made up of multiple inflammatory areas. The centers of these commonly showed varying stages of necrosis. Two types of structure were seen in the necrotic centers, viz., a hyaline eosin-staining material and a fibrillar substance that stained with hematoxylin. Scattered in this necrotic material were varying numbers of polymorphonuclear leucocytes. Surrounding the necrotic centers there were many mononuclear and multinucleated cells (polyblasts) which varied in size and shape. Many of them resembled the epithelioid cells in a tuberculous lesion. These polyblasts generally but not always had a marked tendency to be arranged in a radial or palisade fashion. In this respect the arrangement was similar to that commonly found in the heart valve in acute rheumatic endocarditis. Polymorphonuclear leucocytes were scattered among the polyblasts, in many cases in small pockets or abscesses. These nodules simulate abscesses more closely than the acute rheumatic nodules which we have studied. These necrotic abscessed areas, since they contain streptococci, would probably tend to bring about a state of hypersensitiveness to streptococci in the patient.

The structure of the nodules from chronic arthritis does not differ in any respect except degree from that described in the nodules in subcutaneous tissues, joints, tendons, *galea aponeurotica*, diaphragm, tongue, tonsils, arteries, heart valves, and auricles and ventricles of the heart in cases of acute rheumatic fever.

Seventeen nodules were cultured in beef infusion broth with a reaction of pH 7.6. Streptococci were recovered in pure culture in 12 (70.6%). Some of the strains grew well and some poorly. As a rule they were diplococci, though 2 strains grew out into chains of diplococci. All were gram positive and produced a green discoloration faintly when grown on the sheep blood agar plate for 24 hours at 37°C.

The frequency of subcutaneous nodules in acute rheumatic fever and in chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these 2 conditions, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and subcutaneous nodules in chronic arthritis strongly suggest, for the most part, that acute rheumatic fever and chronic arthritis have a common streptococcic etiology and that the 2 diseases are in all probability different manifestations of the same process.

5980

A Semi-Permanent Acid Hematin Standard for Hemoglobin Determinations with Colorimeter.

L. EARLE ARNOW. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

The standard was prepared as follows: 15.5 mg. of a commercial grade of hemin were dissolved in 50 cc. of 0.1 *N* NaOH. To this mixture was added 25 cc. of a 5% solution of gelatin (to prevent precipitation of the hemin from acid solution),¹ in a liter volumetric flask and made up to the mark with 0.1 *N* HCl. The resulting solution of acid hematin was compared with the Bausch & Lomb brown filter for hemoglobin determination in a Bausch & Lomb colorimeter. The reading was found to be 14.5 mm. Since it was desired that the reading should be 20 mm., 14.5 cc. of the standard were made up to 20 cc. with 0.1 *N* HCl. The resulting solution, placed in the right cup of the colorimeter and compared with the standard slide, gave a reading of 20 mm. A colorimeter cup with fused-on top and bottom 20 mm. apart and side neck for filling² had the side arm drawn out and constricted until an opening of about 2 mm. remained. The standard hematin solution was introduced into this opening by means of a piece of glass tubing drawn out to a capillary point until the cup was completely filled and the side arm about three-fourths full of liquid. The constricted end of the side arm was dried with a piece of filter paper and sealed off. The standard thus prepared matched exactly the standard Bausch & Lomb glass slide. It is believed that the solution prepared as described above will remain standard for about a year, at the end of which time the side arm may be drawn out and reopened, and a new standard introduced into the colorimeter cup.

On the basis of molecular weight calculations it was observed that the commercial hemin sample used was approximately 43% hemin.

¹ Elvehjem, C. A., *J. Biol. Chem.*, 1931, **93**, 203.

² McClendon, J. F., Russell, Sidney, and Tracy, Edward, *J. Biol. Chem.*, 1926, **70**, 705.

New York Section.

New York Academy of Medicine, February 17, 1932.

5981

Experiments on Immunization with Haptens.

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It is known that the specific substance contained in Forssman's antigen which as such is only slightly antigenic¹ can be restored to high antigenic activity by the admixture of proteins foreign to the animal immunized. Recently a second method of producing the same result has been found, which consists in the injection of the hapten along with kaolin.² Similar effects have been described by Zozaya³ with mixtures of a polysaccharide from *B. anthracis*, and collodion particles.

We had no difficulty in corroborating the findings of Gonzalez and Armangué, and in addition we observed that hemolysins for sheep blood could be produced by injecting the Forssman hapten mixed with a suspension of collodion particles. These observations suggested the possibility that other ways of increasing the slight antigenic activity of the hapten might be found. In fact, in some animals we obtained higher titers than in the controls, on injecting the specific substance and at the same time trypan blue or turpentine, but the results were quite irregular. A single attempt to produce precipitins and bacterial agglutinins by Zozaya's method was not successful. However, it was possible to produce agglutinins and precipitins of moderate titer by the injection of a specific substance prepared from *V. cholerae* which was adsorbed to charcoal. This substance was prepared by extraction with 75% alcohol followed by treatment with alkali according to the method of Landsteiner and Levene.⁴ (The sera of 5 rabbits after 5 intraperitoneal

¹ Landsteiner, K., and Simms, S., *J. Exp. Med.*, 1923, **38**, 136.

² Gonzalez and Armangué, *Comp. Rend. Soc. Biol.*, 1931, **106**, 1006.

³ Zozaya, J., *Science*, 1931, **74**, 270.

⁴ Landsteiner, K., and Levene, P., *J. Exp. Med.*, 1927, **46**, 213.

injections of the specific substance from *V. cholerae* adsorbed to charcoal gave, after 2 hours at 37°C., agglutinin titers of (1) 1:1600, (2) 1:100, (3) 1:50, (4) 1:100, and (5) 1:50, respectively. Precipitation reactions, made by adding 2 drops of the immune serum to 0.2 cc. of a 1:5000 dilution of the specific substance were distinctly positive in 2 cases, and weakly positive in 2 others.)

Control animals injected with the substance alone produced no precipitins, and the sera showed only traces of agglutination in a dilution of 1 to 10. A solution of the specific substance still gave a biuret reaction, and on hydrolysis with acid a precipitate was formed which contained alcohol soluble acids.

In one experiment in which an azodye made from diazotized tartrilic acid and resorcinol⁵ was adsorbed to aluminum hydroxide and injected into guinea pigs, a certain degree of sensitization seemed to be brought about, since some of the animals showed mild anaphylactic symptoms and one of them died in acute shock on the injection of a corresponding azoprotein after an appropriate interval.⁵ It was, however, not possible to reproduce this result in a second experiment.

The observations reported show in confirmation of those of Gonzalez and Armangué that the hapten of the Forssman substance can exhibit distinct antigenic activity, and one may reasonably assume that this will hold for other sorts of haptens, without the aid of antigenic proteins. On the other hand, the experiments do not disprove the idea that in the high activity of natural complex antigens the proteins play a significant rôle.

5982

Single Cell Dissociation of the Mycobacterium of "Rat Leprosy."*

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From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York City.

An acid-fast organism isolated by Chapin¹ from a leprous rat in 1912 has been kept in the culture collection of the National Tubercu-

⁵ Landsteiner, K., and Levene, P., *J. Exp. Med.*, 1930, **52**, 347.

* This experiment is part of a group investigation being carried on in conjunction with the Medical Research Board, National Tuberculosis Association.

¹ Chapin, U. S. Treas. Dept., *Pub. Health Rep.*, 1912, **27**, 161.

losis Association for considerable time. Transfers distributed over the country from this and other sources have been designated as "bacillus of rat leprosy", mycobacterium of "rat leprosy", "rat leprosy bacillus", etc. This mycobacterium is probably not identical with acid-fast organisms in tissue from actually leprous rats.^{2, 3, 4, 5} These observers found it very difficult, if not impossible, to cultivate the organisms but stated that the disease could be transmitted from rat to rat by injecting infected tissue. The organism under discussion was received some years ago from the National Tuberculosis Association and labelled "Rat leprosy bacillus No. 368". It grows luxuriantly on Petroff's or Lowenstein's egg medium (pH 7.4) after an incubation of about 4 days. The growth membrane appears deeply orange colored. Growth also takes place on slants of nutrient agar containing no glycerol at pH 7.4, but acid-fast properties are lost after a week of incubation on this medium. Such properties may be regained after planting on the egg media which contain glycerol. The organisms measure from 0.3 to 3.0 mu. in length and about 0.3 mu. in breadth. Numerous granules have been observed on acid-fast and gram-stained preparations. The organism is acid-fast and gram-positive.

After several transfers on petri plates of Petroff's egg medium one of these cultures, initially R in character, was incubated for several weeks at 37.5°C. Upon examination, typical S colonies as well as the usual R colonies were found. The S colony is smooth, round, domed and glistening moist in appearance, and measures $\frac{1}{2}$ to 2 mm. in diameter, while the R colony is flat, dry, dull and rough and wrinkled in configuration, measuring some 2 to 4 mm. in diameter. The S colony makes a homogeneous suspension in normal sodium chloride solution or distilled water; the R colonies, on the contrary, flocculate and precipitate even after prolonged shaking. These 2 colony types are distinct and clear-cut.

Several representative R and S colonies were then selected and by applying Petroff's⁶ filtering technic a number of plates were obtained from each, having widely separate colonies. These colonies for the most part bred true to type but on some plates of the S colonies, R types were found to have grown, while on one of the plates seeded with an R colony suspension 3 distinct S colonies were noted. These

² Stefansky, *Centralbl. f. Bakteriol.*, 1903, **33**, 481.

³ Dean, *Centralbl. f. Bakteriol.*, 1903, **33**, 222; *J. Hyg.*, 1905, **5**, 99.

⁴ Wherry, *J. Am. Med. Assn.*, 1908, **1**, 1903.

⁵ Zinnser and Carey, *J. Am. Med. Assn.*, 1912, **53**, 507.

⁶ Petroff, *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 632.

S colonies from an initial R form, and R forms from an initial S colony again, developed after a subsequent reculturing of the second dissociants. Obviously, either the homologous colony was not homogeneous for type or spontaneous dissociation was taking place.

To answer this question several single bacteria were isolated from both the R and S types of colonies. The single bacterial cells were allowed to form micro-colonies in the micro-culture after which the resultant growth was drawn into a micro-pipette and expelled upon a slant of Petroff's egg medium. By allowing a micro-colony to form in the microscopic drop containing a single bacterial cell before any attempt is made at transplantation, a much higher percentage of successful cultures is obtained from single cells than if the freshly isolated single organism is planted directly. The details of this technic will be published later.

The cultures obtained from single organisms of both the R and S types of colonies bred true for 3 or more generations, giving rise only to their homologous colony forms. Sooner or later, however, in both instances dissociation unquestionably occurred. Typical R colonies grew on plates receiving inoculation with an actual S colony from a single cell S strain and S colonies developed on plates which were seeded with an actual R colony from an R single cell strain. The mutation is somewhat more prolific from S to R, but the R to S change may not be termed at all infrequent, as is the case among many other species of bacteria. The dissociation of these single cell strains took place spontaneously, no special effort being made to induce it. This confirms observations on single cell cultures of avian *Mycobacterium tuberculosis*.⁷

5983

**Relative Immutability of Hydrogen Ion Concentration of the Bile.
Its Buffering Effect in Bactericidal Experiments.**

REUBEN OTTENBERG AND JOSEPH KAHN.

From the Laboratories of Mount Sinai Hospital, New York.

In 1926 we noticed that solutions of phenol-tetra-chlor-phthalein and phenol-tetra-iodo-phthalein are decidedly bactericidal for typhoid bacilli. These dyes, however, when concentrated in the gall-bladder of animals after intravenous injection (Abel and Rown-

⁷ Kahn, Proc. 33rd Ann. Meeting Soc. Am. Bact., 1931, 10.

tree¹) show no bactericidal effect whatever. In simple experiments we found that the bactericidal effect of aqueous solutions of these dyes was due entirely to their alkalinity. As excreted, however, they seem to be in a colloidal state and have no effect on the reaction of the bile.

We wished, therefore, to find out whether it was actually possible to make the bile more alkaline. A Rous fistula² was established in a dog, the bile collected and examined daily by potentiometer. It was impossible to produce an appreciable change in the reaction of the 24 hour bile by administration of large doses of sodium bicarbonate by mouth or intravenously, or by intravenous administration of the very alkaline solution of phenol-tetra-chlor-phthalein (pH 9.52). (Protocol I.) There was not even a transient increase in alkalinity of bile after the injections; a specimen collected for 6 hours after an intravenous injection of bicarbonate of soda showed a change toward the alkaline side too small to be of significance.

PROTOCOL I.

Dog 1 Male, 10 kilo wt. Procedure			Dog 2 Female, 9 kilo wt. Procedure		
	pH of 24 hour bile	Day after operation	pH of 24 hour bile	Amt. of bile in 24 hrs.	
After 10 gm. NaHCO ₃ by stomach tube	8.65	2	8.30	60 cc.	
	8.90	3	8.60	55	
	8.92	4	8.55	70	
	8.69	5	8.45	70	After 20 gm. NaHCO ₃ by stom- ach tube
		6			
		7	8.35	(2 days) 210	
After 12 gm. NaHCO ₃ by stomach tube	8.66	8	8.36	110	
		9	8.40	90	
1.5 gm. NaHCO ₃ (25 cc. of 5% solution	8.75 (6 hr. spec.)	11	8.40 (4 hr. spec.)		30 gm. NaHCO ₃ by stomach tube*. Spec. examined, 10 cc. col- lected 4 hr. after bi- carbonate adminis- tration
Intravenous injection of 5 cc. of 5% phenol-tetra- chlor-phthalein	8.45 8.60 8.60 8.60	15 16 22 23			

* Vomited a small amount after 1½ hours.

¹ Abel and Rowntree, *J. Pharm. and Exp. Therap.*, 1909, **1**, 231.

² Rous and McMaster, *J. Exp. Med.*, 1923, **37**, 11.

Recently we became interested in attempts to cure typhoid carriers. Nichols,³ hoping to cure typhoid carriers, attempted to make the patient's bile more alkaline by administration of large doses of sodium bicarbonate by mouth. (Typhoid bacilli are easily killed by alkaline solutions.) Some favorable results were reported. Krause⁴ states that "change of reaction by administration of acids or alkalis has so far given no results".

We therefore repeated our earlier animal experiment to make the bile more alkaline. We found it was impossible to make the bile more alkaline. (Protocol I, Dog 2.)

Duttman⁵ gave large doses of alkalis or acids by mouth to patients with temporary biliary fistulas. He found only negligible changes of hydrogen ion concentration of the bile. We have confirmed Duttman's results in experiments on 3 patients convalescent after the operation of cholecystectomy with drainage of the common bile duct. The daily drainage of bile was collected with sterile precautions and its hydrogen ion concentration determined by potentiometer. Oral administration of as much as 20 gm. of sodium citrate or 24 gm. of sodium bicarbonate or of 6 cc. of dilute hydrochloric acid failed to cause any significant change in the pH of the bile (Protocol II).

PROTOCOL II.

Day after operation	Patient	Administered to patient (<i>per os</i>)	pH of Bile		
			Pat. A	Pat. B	Pat. C
2			8.11	8.61	8.30
3			8.07	8.53	8.20
4	A	Sodium citrate 4 gm. every 4 hr. 5 doses. 24 hr. bile	8.11	8.30	7.84
5				8.20	
6	B	NaHCO ₃ 12 gm. in 1 hr. (divided doses). Bile in 3 hr. after bicarbonate	8.31	8.62	8.04
7	A	NaHCO ₃ 4 gm. every 4 hr. for 6 doses. 24 hr. bile	8.20	8.61	
	C	Sodium citrate 12 gm. within 1 hr. 3 hour bile collection			8.14
8				8.62	7.80
9	B, C	Dilute HCl 6 cc., divided doses, 1 hr. 3 hour bile collection		8.56	7.87
			Bile very dark	Bile throughout very dark viscid	Bile throughout rather pale and fluid

³ Nichols, *J. Am. Med. Assn.*, 1917, **68**, 958.

⁴ Krause, *Monatschr. f. Kinderheilkunde*, 1929, **44**, 358.

⁵ Duttman, Bruns, *Beitr. z. klin. Chir.*, 1927, **139**, 720.

It is interesting to note that the bile of each dog and of each patient tends to range about a somewhat characteristic average pH. The bile of the dogs was sterile or nearly so. That of the patients was undoubtedly infected to some degree. This may perhaps explain the greater variability of the patient's pH.

The bactericidal effect of alkali added directly to bile is nullified by the buffering effect of bile. This was shown by the following experiment. In 2 rows of tubes graded quantities of N 1/10 sodium carbonate (0.1, 0.2, 0.3, 0.4, 0.5 cc.) were diluted to equal volume (1.5 cc.) using as diluent in the first row 1 cc. of sterile (boiled) human fistula bile and a small amount of water, in the second row, water only. Then 0.1 cc. of diluted typhoid culture in bouillon was added to all the tubes. After one hour incubation at 37°C. the contents of all were plated in agar. Even the largest amounts of sodium carbonate, when mixed with bile, failed to show any bactericidal effect, while even the smallest amount showed some bactericidal effect in water.

This effect is not caused by any protective effect of bile for the bacteria. We have tested bile with a number of antiseptics, HgCl_2 , mercurochrome, salyrgan, and others, and found it only to interfere slightly with their bactericidal power for typhoid bacilli. We have commenced work on the titrimetric buffer effect of bile.

Summary. It thus appears that (1) alkalis given by mouth or vein and hydrochloric acid by mouth produce hardly any change in the hydrogen ion concentration of secreted bile and (2) the bactericidal effect of alkaline solutions is destroyed by addition of bile. (3) Increased alkalization of bile (at least by the methods used) does not occur. The great stability of the hydrogen ion concentration of bile is a fact of considerable interest.

This observation, together with the well recognized fact that increased fluid intake does not increase the volume of bile secreted^{6,7,8} suggests that bile secretion is relatively independent of those conditions of water and electrolytes in the blood which so greatly influence the secretion of urine.

⁶ Westphalen, 1873.

⁷ Gundermann, *Beitr. z. klin. Chir.*, 1923, **128**, 1.

⁸ Snell and Rowntree, *Am. J. Physiol.*, 1928, **85**, 577.

5984

Models Showing Accumulation.

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Models consisting of a non-aqueous layer separating 2 aqueous solutions (the inner being more acid than the outer) show the following points of resemblance to *Valonia*:

(1) Penetration from the outer into the inner aqueous solution (artificial sap) is in the following order $K > Na > Mg > Ca$. Also $Cl > SO_4$.

(2) Instead of equilibrium a steady state is reached in which the artificial cell "sap" increases in volume but remains approximately constant in composition.

(3) Osmotic pressure and K-concentration become greater inside than outside.

(4) In certain models the "sap" contains CO_2 . As potassium enters and $KHCO_3$ is formed the osmotic pressure rises. Although CO_2 is regarded as a waste product in organisms it acts in this case to increase the energy stored in the cell.

5985

Anginal Syndrome Induced by Gradual General Anoxemia.

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With individuals subject to attacks of chest pain, in whom there is no evidence by physical or electrocardiographic examination of myocardial (coronary) disease, it is often difficult to be certain of the origin of the pain. We wished to distinguish between those with pain due to impaired coronary circulation, and those in whom the pain arose otherwise. It occurred to us that if one were to produce a general anoxemia, and, therefore a local cardiac anoxemia, there might appear differentiating responses in these 2 groups.

By means of rebreathing, we were able to produce a state of general anoxemia in human subjects. The carbon dioxide was absorbed. It usually took about 10 minutes for the oxygen to become so low that the patient became uncomfortable.

Twenty-seven patients were subjected to the test. Fourteen patients were used as controls. The controls consisted of 4 patients with normal hearts. The remaining cases were patients with chronic valvular disease, paroxysmal auricular fibrillation, rheumatic fever, spondylitis, gall bladder disease, and cardiospasm. None of these patients developed pain. Thirteen were patients with clear-cut histories of attacks of precordial pain brought on by exertion, excitement, eating, or exposure to cold. Ten developed pain during the rebreathing test. Eight of these had no physical or electrocardiographic signs of myocardial disease. The pain appeared when the oxygen fell to about 9 to 10%. This ordinarily took about 8 to 10 minutes. Subjects were advised to raise their hands when they felt uncomfortable, and the experiment was stopped.

It is furthermore interesting to observe that 2 other patients with clinical angina and intra-ventricular block developed pain and additional electrocardiographic changes during the anoxemia. The changes found were depression of the R-T segments in Leads II and III, and in another instance in Leads I and III. Unfortunately before further tests could be made, the second patient died, but the patient with changes in II and III was observed further. We were unable to reproduce the electrocardiographic changes with (1) oxygen and carbon dioxide inhalations, (2) intravenous atropin, (3) adrenalin, (4) pitressin, and (5) amyl nitrate.

Three patients with clinical coronary artery disease, one with definite electrocardiographic changes, and two with no electrocardiographic changes, responded negatively to the test.

We feel, therefore, that it is possible to reproduce the pain of angina pectoris in susceptible subjects by inducing general anoxemia. We assume in the explanation that the coronary circulation, normally adequate, becomes inadequate during anoxemia. We are not prepared to state that anoxemia *per se* is responsible for the pain that appears during the experiment. It is possible that the explanation of angina as being due to a cardiac ischemia combined with a piling up of Lewis's P-factor explains the pain that appears during rebreathing. We feel that our work, although not conclusive, tends however to support the anoxemia theory of angina pectoris. This test promises to be of some value in differentiating the causation of chest pain.

5986

Attempts at Treatment of Hemorrhagic Diathesis by Injections of Snake Venom.

SAMUEL M. PECK. (Introduced by Louis Gross.)

From the Laboratories of the Mount Sinai Hospital, New York City.

In 1930 the author attempted to produce a Schwartzman phenomenon (local hemorrhagic necrosis following local and intravenous injections of bacterial toxic filtrates with fungus extracts). These efforts were unsuccessful. In the hope that previous local injections of a vascular poison might render the reaction site more sensitive to hypothetical toxic substances in fungus cultures, snake venom was employed. This again proved unsuccessful. Later, in collaboration with Dr. Harry Sobotka,¹ the author showed that if 14 days to one month were allowed to elapse between the time of injecting of rabbits with snake venom and the elicitation of a Schwartzman phenomenon, a large number of the animals became refractory to the latter. Since no circulating antibodies could be demonstrated to explain the refractory state, and since antivenin had no effect on the course of the Schwartzman phenomenon, the induced refractory state was thought to be due to some change in the vessel walls which made them resistant to toxic filtrates and this prevented the hemorrhage. With this possibility in mind, the author then treated with snake venom diseases which can be grouped under hemorrhagic diatheses. Patients showing certain allergic phenomena such as urticaria, neurodermitis, as well as asthma and hay fever were also treated.

Clinical trial was made in 44 cases which can be divided roughly into 3 groups:

Group I consists of 3 cases of thrombocytopenic purpura,* one case of Henoch-Schönleins syndrome (Frank's capillary toxicosis), and 2 cases of hemophilia. The hemorrhagic purpura cases had such symptoms as epistaxis and purpura; and in 2 of them there was a history of prolonged and profuse menstrual periods. Two of this group have been under treatment with snake venom for 9 months, and one for 6 months. The 3 patients showed definite clinical improvement with a marked decrease in purpura and epistaxis, and a decrease in the duration and profuseness of the men-

¹ Peck, S. M., and Sobotka, H., *J. Exp. Med.*, 1931, **54**, 407.

* Since submitting this report for publication two additional cases of thrombocytopenic purpura have been treated with similarly good results.

strual flow. In one patient the platelet count is now 220,000, while in the other 2 it is still below 60,000.

The 2 hemophiliacs have been under observation by Dr. N. Rosenthal for a number of years. They have now been under snake venom treatment for 5 months with what appears to be encouraging results.

The patient with Henoch-Schönleins syndrome had recurrent purpura, hematuria and a history of prolonged and profuse menstrual periods. The menstrual period which came on after she had received treatment with snake venom for several weeks was shorter and markedly less profuse. Her hematuria has disappeared and she has been free from purpuric manifestations for over a month.

Group II, in which the best results have been observed, consists of a case of epistaxis (idiopathic) present for more than a year in a 7-year-old boy, and 5 cases of functional uterine bleeding also of long duration. Very soon after treatment was begun these cases showed a definite improvement in the form of a reduction in duration and amount of bleeding. It is impossible as yet to determine whether the results are permanent. These cases will be reported in detail with Dr. M. Goldberger.

Group III includes 32 allergic cases of the type previously mentioned. In the majority of them the results have not been satisfactory. In a few cases, however, very definite clinical improvement seemed to follow the treatment.

Method. The venom used was that of the moccasin snake (*Ancistrodon piscivorus*). The standard dose for an adult was 0.2 cc. of a 1:3000 solution in physiological saline given intradermally. This was increased in most cases to 2 injections of 0.2 cc. each in different sites. The injections were given once or twice weekly, and the duration of the treatment varied with the cases.

Comment. The clinical results in cases with such varied etiologic factors seem to substantiate the theory that the venom achieves its results by acting directly on the blood vessels. This viewpoint is strengthened when we consider that there was a marked beneficial effect on the tendency to hemorrhage in 2 of the cases of thrombocytopenic purpura, although the platelet count did not rise appreciably.

Stockton and Franklin² have reported good results with antivenin in a case of purpura. Antivenin has had no therapeutic effect in one of the author's cases of thrombocytopenic purpura.

Experiments in rabbits seem to show that in these animals anti-

² Stockton, M. R., and Franklin, C. H., *J. Am. Med. Assn.*, 1931, 96.

venin plays no rôle in the production of the resistant state to bacterial toxic filtrates produced by the venom.

5987

Effect of the Prolonged Use of Ethyl Alcohol on Renal Function and Pathology in the Dog.*

WM. DE B. MACNIDER AND G. L. DONNELLY.

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Observations were previously made on the acute toxic effect for the kidney in both normal and naturally nephropathic dogs of an alcoholic distillate obtained after the fermentation by yeast of a mixture of corn meal and sugar or corn meal and molasses.¹ It was later demonstrated² that such distillates were able to induce a type of chronic nephropathy in the dog which was largely confined to the endothelium of the glomerular capillaries. The animals in the 2 groups of experiments were given 10 cc. per kg. of the distillate. Normal dogs were used as controls and received 10 cc. per kg. of a 40% solution of ethyl alcohol. The experiments lasted from 6 weeks to 3 months. The animals receiving the alcoholic distillate as well as the control animals were then sacrificed. Both the normal and naturally nephropathic dogs which received the alcoholic distillate showed definite evidence both functionally and anatomically of renal injury. The control dogs gave but slight evidence in so far as renal function was concerned of any departure from the normal. Similar observations of the relative nontoxicity of ethyl alcohol for pigeons have recently been made by Hanzlik.³

The present investigation is concerned with the effect of ethyl alcohol on renal function and pathology in the dog when taken over long periods in the amount of 10 cc. per kg. of a 40% solution. The experiments have lasted from 6 months to 2 years and 2 months. Twenty-three adult dogs have been used. The animals were kept in metabolism cages and allowed an unrestricted amount of water except on the days set aside for observation. They were fed on bread, scraps of meat, a small amount of butter and milk.

* This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

¹ MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1925, **26**, 97.

² MacNider, Wm. deB., *Proc. Soc. Exp. Biol. and Med.*, 1925, **22**, 52.

³ Hanzlik, P. J., *J. Pharm. and Exp. Therap.*, 1931, **43**, 339.

The solution of ethyl alcohol was given once a day before the morning meal. For 3 days prior to the day on which observations were made, the animals were given 300 cc. of water twice a day in order to insure a constant water intake. At such periods the urine was studied qualitatively for the presence of albumin, glucose, acetone, and diacetic acid. Centrifugalized portions of urine obtained by catheterization were examined for casts. The elimination of phenolsulphonephthalein over a 2 hour period was determined by the method of Rowntree and Geraghty. Blood urea and non-protein nitrogen estimations were made by the methods of Folin and Wu. The reserve alkali of the blood was determined by the method of Marriott.

The results are as follows:

1. The use of ethyl alcohol in the amount and percentage indicated, induces for a period which has varied in the respective animals, a state of semiintoxication characterized by muscular incoordination especially of the hind limbs and a staggering gait which is followed by drowsiness and usually by a period of sleep. Twelve of the dogs after having received the alcohol for periods which varied from 4 to 7 months failed to show any of the above mentioned symptoms. The remaining 11 animals gave evidence of such an intoxication until the experiments were terminated. The development of a tolerance to alcohol in so far as the effect on the central nervous system is concerned is variable and suggests that in those animals which fail to develop a tolerance some factor other than the alcohol may be concerned in this variation.

2. Nineteen of the 23 dogs showed an increase in body weight, varying from 3.75 kg. in an animal that had received alcohol for 6 months and 10 days to 6.32 kg. in an animal receiving the alcohol over a period of $7\frac{1}{2}$ months. The dogs were presumably full grown at the commencement of the experiments, aged from $3\frac{1}{2}$ to 7 years. The increase in body weight cannot be attributed to the natural processes of growth nor to the length of time over which the animals were subjected to the use of alcohol. Six animals that received the alcohol for 2 years and 2 months increased less in weight than did 17 of the dogs which received alcohol for a shorter length of time. Of the 19 animals which gained in weight 12 showed both acetone and diacetic acid in the urine and 8 developed a glycosuria.

3. The influence of ethyl alcohol on urine formation was ascertained by the following procedure. After a period of 3 days during which the dogs were given 300 cc. of water twice a day by

stomach tube, they were given on the fourth day the normal dosage of alcohol in a sufficient amount of water to bring the total volume up to 300 cc. During a 3 hour period following the use of alcohol in such a dilution there was an increase in the output of urine by all the animals over the amount formed on the 3 preceding days when the volume of fluid remained constant. The average increase in urine formation by all of the dogs varied from a minimum increase of 84 cc. by the animal of Exp. 6, to a maximum increase of 211 cc. by the animal of Exp. 21. The diuretic effect of alcohol cannot be solely explained on the basis of the associated water intake.

4. Eighteen of the 23 dogs developed an albuminuria, varying from a faint trace of albumin to 1.6 gm. per liter. Seven of the animals within 6 hours after the intake of alcohol showed albumin varying from 0.5 to 1.6 gm. per liter. With the exception of 2 of these dogs the albuminuria was transitory and disappeared within 24 hours. Any subsequent use of alcohol resulted in its recurrence. The observation is interpreted as a transitory functional disturbance not dependent upon structural changes in the kidney. The histological study of the kidneys of such animals showed no abnormality other than an increase in stainable lipoid material in the cells of the ascending limb of Henle's loop. These 7 animals which reacted to the use of alcohol by the development of more than a trace of albumin, a definite albuminuria, also showed a glycosuria, the presence of acetone and diacetic acid in the urine, a high elimination of phenolsulphonephthalein which varied during a 2 hour period from 72 to 96% and a reduction in the reserve alkali of the blood from the normal of 8.0 or 8.1 to a maximum reduction of 7.85. There was no retention of urea or non-protein nitrogen.

The presence of casts in the urine of those animals showing only a trace of albumin was variable. In the group developing an albuminuria which varied from 0.5 to 1.6 gm. per liter, narrow hyaline casts were constantly present. With the disappearance of the albuminuria the number of casts was first reduced and gradually disappeared. This sequence of events would again indicate a functional rather than a structural disturbance in the kidney.

5. The elimination of phenolsulphonephthalein has been very variable when contrasted with the output of this dye before the administration of alcohol. The normal elimination of this substance over a 2 hour period has varied from a minimum output of 58% to 71%. There was no constant change in the time of appearance of the dye in the urine. The variations in the elimination of phenolsulphonephthalein by the respective animals have developed

within the first month after the use of alcohol was commenced. It must therefore be attributed to some rather acutely developing disturbance. In 8 of the 23 dogs there was a persistent reduction in the elimination which continued without much change throughout the experiments. The percentage reduction in the following 2 animals is indicative of this type of modification in the total output of the dye. The animal of Exp. 2 had a normal elimination of 58% which within a month was reduced to 41% and at the termination of the experiment one year and 8 months after the commencement of the alcohol was 36%. The animal of Exp. 19 had a normal phenolsulphonephthalein elimination of 67% which was reduced within a month to 52% and at the end of the second year when the experiment was terminated the output was 46%.

In a second group of 8 animals the elimination of the dye was practically unaffected by the use of alcohol. The normal elimination of the dye by the dogs in this group varied from 59 to 71%. The output during the course of the experiments varied from 50 to 64%. In a third group of 7 dogs which has been previously referred to, the elimination of the dye was definitely increased after the commencement of the use of alcohol. This increase in elimination above the percentage which these animals had established as their normal percentage elimination depended to some extent on the relationship between the time at which the estimations were made after the alcohol had been administered. The most marked increase in the output of the dye occurred when the determinations were made within 3 hours after the injection of alcohol. If the use of alcohol was withheld for 48 hours there developed a reduction in the elimination of the dye. This observation would indicate the occurrence in certain of the animals of either a functional disturbance in the mechanism concerned in the elimination of the dye by the organism or the development of some structural defect which could be readily repaired. The following experiments illustrate the increased elimination of the dye by the animals falling in this third group. The normal elimination of phenolsulphonephthalein by the animal of Exp. 14 was 60%. During the course of the first month of the experiment there developed an increased output to 78%. In the animal of Exp. 19 the output of the dye increased from 62% to 96%. In this group of animals that have shown an increase in the elimination of phenolsulphonephthalein there has also occurred an albuminuria with casts, a glycosuria, the appearance of both acetone and diacetic acid in the urine and a reduction in the reserve alkali of the blood. There was no retention of urea or non-protein nitrogen.

6. The alkali reserve determinations of the blood have been normal and have varied from 8.0 to 8.1. These determinations have remained within the normal during the course of the experiments with the exception of the 7 dogs which have shown an increase in the elimination of phenolsulphonephthalein and certain abnormalities in the composition of the urine.

At the termination of the experiments both liver and kidney tissue were immediately fixed in Zenker's solution and in corrosive-acetic, embedded in paraffine and stained with eosin and haemotoxylin and with eosin and methylene blue. Tissue for frozen sections was fixed in 10% formalin and stained for lipid material by Herxheimer's method with Scharlach R. The present study is concerned only with the changes developing in the kidneys. The kidneys of these animals regardless of the duration of the experiments have failed to show any gross surface changes. The capsules have not been adherent. The cortical substance has been smooth without scars. On section the kidneys have cut without a feeling of resistance and there has been no change in the volume relationship between the cortex and the medulla. In 9 of the dogs the kidneys showed a definite, apparently fatty zone at the junction of the cortex and medulla. The microscopic study has very largely substantiated the gross findings. The glomeruli have in general appeared normal without any very definite capsular or intracapillary changes. In 11 of the animals these structures appeared to show an increase in the number of endothelial nuclei which stained intensely and in such glomeruli the basement membrane of the capillaries was thickened. Stainable lipid material was present as fine dust-like granules in the endothelium of the capillaries in 4 of the animals. The epithelium of the tubules has shown no change of an advanced degenerative character. Edema, vacuolation and necrosis of such cells have not been observed. The most constant change in the epithelial tissue of the kidney has been an increase in the amount of stainable lipid material in the cells of the ascending limb of Henle's loop. In such a location this material may accumulate to an extent as to obliterate the outline of the cells and obscure their nuclei. Those animals which have shown such a marked accumulation of lipid material in this part of the tubule have also shown smaller droplets and dust-like particles of the same material in the cells of other segments of the tubule. There has been no definite increase in intertubular connective tissue.

Conclusions. 1. Ethyl alcohol when given daily to dogs over long periods in an amount sufficient to induce symptoms of alco-

holic intoxication is but slightly nephrotoxic in a structural sense. The evidence of structural changes in the kidneys has consisted in certain early changes in the glomeruli which have not been of constant occurrence and by an accumulation of stainable lipid material in the epithelium of the tubules. 2. The experiments indicate that a functional disturbance may be induced in the kidney from the use of alcohol which is not dependent upon microscopically demonstrable structural changes. Such a disturbance has been characterized by an increase in urine formation and in a certain number of the animals by an albuminuria with casts. The elimination of phenolsulphonephthalein has been variable. Such alterations in renal function other than a persistent decrease in the elimination of phenolsulphonephthalein tend to disappear with the discontinuance in the use of alcohol.

5988

Relationship Between Liver Injury Induced by Alcohol and Elimination of Phenolsulphonephthalein.*

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In the preceding publication¹ concerning the nephrotoxic action of ethyl alcohol when administered over long periods to dogs, the observation was made that the elimination of phenolsulphonephthalein was variable. On the basis of this variation the 23 animals studied could be divided into 3 fairly well defined groups. The first group showed a moderate reduction in the output of the dye persisting throughout the experiments. In a second group, the elimination was but slightly affected, while in a third group of 7 dogs the elimination of the dye was definitely increased following the commencement of the use of alcohol. The most marked example of such increase occurred in Experiment 19, in which the percentage output of the dye rose from 62 to 96%. The dogs which showed such a change in the elimination of phenolsulphonephthalein also developed an albuminuria with casts, glycosuria, appearance of both acetone and diacetic acid in the urine and reduction in the acid-base equili-

* This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

¹ MacNider, Wm. deB., and Donnelly, G. L., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 581.

brium of the blood. There was no retention of urea or non-protein nitrogen. Histological studies of the kidneys of these animals afforded no anatomical basis for the apparent contradiction of an albuminuria with casts, no retention of urea and non-protein nitrogen and an increase in the elimination of phenolsulphonephthalein.

Rowntree and Geraghty² while studying the elimination of phenolsulphonephthalein in various pathological states of the kidney observed that in some of the intoxications occurring during pregnancy, the output of the dye was definitely increased. Later, Whipple, Pieghal and Clark³ and very recently Hanner and Whipple⁴ have shown a relationship to exist between a liver injury and an increase in the output by the kidneys of phenolsulphonephthalein.

As a result of the observation of the increase in the elimination of this dye in certain of the animals intoxicated by alcohol, histological studies were made of the livers of such animals and of those animals in which there was either no appreciable change in the output of the dye or in which the elimination of it was reduced. On this basis the animals used in the previous study¹ may be divided into three groups. The animals falling in Groups 1 and 2 in which the elimination of the dye was either not affected by the use of alcohol or only slightly reduced are used as control animals for Group 3, represented by 7 dogs in which the elimination of the dye was definitely increased. In Group 3 the increase in the elimination of phenolsulphonephthalein developed within the first month after the commencement of the use of alcohol and was most marked when the determinations were made within 3 hours after its injection. If the use of alcohol was withheld for 48 to 72 hours the initial increase in the output of the dye underwent a reduction. In 2 of the dogs its elimination returned to the normal percentage elimination established before the commencement of the experiments. The following experiments are characteristic of the alterations in the elimination of the dye by this third group of animals. The dog of Experiment 2 had a normal output of the dye of 64%. During the first month of the alcoholic intoxication the percentage elimination increased to 82%. In the animal of Experiment 19, the normal output was 62%, which increased to 96%. In the dog of Experiment 4, the normal elimination was 58%, which following

² Rowntree, L. G., and Geraghty, J. T., *J. Pharm. and Exp. Therap.*, 1909, **1**, 579.

³ Whipple, G. H., Pieghal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1909, **20**, 278.

⁴ Hanner, J. P., and Whipple, G. H., *Arch. Int. Med.*, 1931, **48**, 598.

the use of alcohol increased to 72% and with the withdrawal of the alcohol decreased to 61%. In the dog of Experiment 14, with a normal elimination of 49%, the use of alcohol caused an increase to 60%. With discontinuance of alcohol the output was reduced to 48%, 2% less than the animal's normal elimination.

Liver tissue from the 23 dogs was fixed by Zenker's fluid and in a solution of corrosive-acetic, embedded in paraffine and sections stained with eosin and haematoxylin and with eosin and methylene blue. Tissue for frozen sections was fixed in 10% formaline. The histological study of such tissue from the animals of Group 3 which had developed a definite increase in the elimination of phenolsulphonephthalein showed a marked liver injury which first involves the periphery of the liver lobule and extends inward towards the

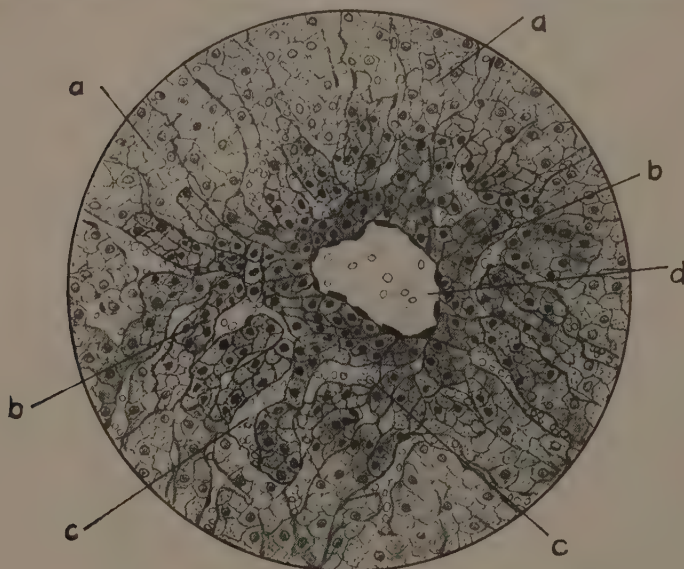


FIG. 1.

Camera lucida drawing, Zeiss, oc. 20X, obj. 40. The figure is from the liver of the animal of Exp. 19. The animal had a normal elimination of phenolsulphonephthalein of 62%. Following the use of alcohol and associated with the development of the liver injury the output of the dye increased to 96%. The figure represents a liver lobule which at a, shows in its periphery and extending inward towards the dilated central vein d, liver cells in an advanced stage of cloudy swelling. The nuclei are small and stain faintly. Such edematous cells contain a large amount of stainable lipid material. The liver cells at b, immediately around the central vein and extending for a distance towards the inner third of the lobule show less swelling and the nuclei stain intensely. In such cells the amount of stainable lipid material is greatly reduced. At c, intralobular capillaries are shown distended with blood.

central vein. By such an extension more than two-thirds of the liver lobule may participate in the pathological process. The changes in such areas of degeneration consist in a very marked cloudy swelling of the liver cells which is usually not accompanied by vacuolation or necrosis. The nuclei of such cells are small in proportion to the amount of surrounding cytoplasm and stain faintly. Their outline is usually well preserved. In such edematous cells there has occurred a marked accumulation of stainable lipid material. As the center of the liver lobules is approached these changes diminish in their severity until immediately around the central vein the liver tissue appears normal. The intertubular capillaries are distended with blood. Hemorrhage into the liver substance has not been observed. There has been no increase in connective tissue. Fig. 1.

The study of liver tissue from the control groups of animals, Groups 1 and 2, in which the elimination of phenolsulphonephthalein was not increased has failed to show this characteristic change in the liver lobule. The livers of such animals have shown an increase in the amount of stainable lipid material over the amount which can be demonstrated in normal animals that have not been subjected to the use of alcohol. Its distribution is of a diffuse character throughout the lobule and is not especially marked at the periphery and central portions of the lobule. Such cells may show an increase in size from an early cloudy swelling. This change has also failed to show the characteristic localization in its severity, to first, the peripheral part of the lobule with an extension towards the central vein.

Conclusions. 1. The foregoing observations of the changes which develop in the liver in those animals which show an increase in the elimination of phenolsulphonephthalein in the urine from the use of alcohol when contrasted with animals which do not show these changes, would indicate that the liver injury is responsible for the increased output of the dye by the kidney either by decreasing the excretion of the dye by the liver into the intestine with its reabsorption and elimination by the kidney (Rowntree and Geraghty²), or by decreasing the ability of the liver to destroy it (Kendall³). These results obtained from the use of alcohol in 7 of 23 dogs, coincide with the observation of Hanner and Whipple⁴ on the relationship which exists between a liver necrosis induced by chloroform and an increase in the elimination of phenolsulphonephthalein.

2. In this series of animals which developed a liver injury from

³ Kendall, E. C., *J. Am. Med. Assn.*, 1917, **68**, 343.

alcohol with an increase in phenolsulphonephthalein elimination the observation was made, that if the use of alcohol was withheld from 48 to 72 hours the elimination of the dye underwent a reduction. An investigation of the liver changes which may develop in connection with the decrease in the output of this dye is now in progress.

5989

Evidence of Physiological Mutations in the Cladoceran, *Moina macrocopa*.

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Extensive breeding experiments with *Daphnia longispina* (Banta and Wood,¹ and unpublished data) give rather conclusive evidence that recessive mutations having physiological effects accumulate during parthenogenesis in this species. In order to learn whether recessive physiological mutations accumulate similarly in *Moina macrocopa*, 2 strains of this species were inbred (sexual reproduction). One strain, Banta's Line 1012, had a laboratory history of 1090 parthenogenetic generations before inbreeding, whereas the other line, Banta's Line 1705, had only a short history of 42 parthenogenetic generations before inbreeding. Of 151 ephippial (sexual) eggs obtained from Line 1012, 118 or 78.1% hatched. Of 321 ephippial eggs obtained from Line 1705, 282 or 87.9% hatched. Eggs in both of these groups include several series which fairly consistently showed the same differential rate of hatching as is represented by the totals given above. This is suggestive of the accumulation during long-continued parthenogenesis of semi-lethal mutations in Line 1012.

Fifty-five females which hatched from the sexual eggs obtained by inbreeding Line 1012 were compared with 55 parthenogenetically-produced females of Line 1012 as to their viability, reproductive capacity, and other characteristics.* Comparisons were made be-

¹ Banta, A. M., and Wood, T. R., *Z. ind. Abs. Vererb.*, Supplementband I, 1928, 391.

* Inasmuch as there is no reduction division in the maturation of the parthenogenetic egg of Cladocera, all parthenogenetically produced descendants of an individual should be genetically identical (except as a mutation may occur). In sexual reproduction, on the other hand, segregation and recombination of genetic factors are, naturally, to be expected.

tween the individuals of these 2 groups, on the basis of the following specific points: early mortality (died before maturity), sterility (attained maturity but died without producing young), longevity, number of broods of parthenogenetic young produced, and reproductive index (average number of young per mother per day of reproductive life). Of the sexually produced individuals 13 died early and 2 were sterile; only 1 of the parthenogenetically produced individuals died early, though 5 were sterile.

TABLE I.

	Longevity in Days																			Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Sexually produced	1	7	5	1	1	2	2	1	1	4	1	4	2	7	3	7	4	0	2	55
Parthenogenetically produced	0	0	1	1	1	4	3	2	2	5	3	11	6	5	6	4	1	0	0	55

TABLE II.

	Number of Broods Produced										Total
	1	2	3	4	5	6	7	8	9	10	
Sexually produced	2	2	2	6	4	7	6	6	2	2	39
Parthenogenetically produced	3	2	5	7	15	9	5	2	1	0	49

TABLE III.

	Reproductive Index																
	5	6	7	8	9	10	11	12	13	14	15	16	17	Total			
Sexually produced	1	0	2	0	4	2	3	5	3	5	6	2	0	33			
Parthenogenetically produced	0	0	0	0	0	0	5	5	5	6	10	6	2	39			

Table I shows the longevity of the sexually produced and the parthenogenetically produced individuals. The longevity ranged from 1 to 19 days. The sexually produced animals died earlier. In Table II the individuals studied are classified according to the number of broods which they produced. Tables I and II show that there is a greater variability among the sexually produced individuals than among the parthenogenetically produced animals in regard to longevity and number of broods produced.

Table III shows the individuals arranged according to their reproductive indices, the range being from 5 to 17. In computing the reproductive index only such as had produced 4 broods or more were considered, inasmuch as *Moina macrocopa* individuals ordinarily live to produce 4 broods or more, and a reproductive index computed from a smaller number of broods would appear to have little significance. The reproductive indices for the sexually produced individuals spread over a rather wide range, whereas the re-

productive indices of the parthenogenetically produced individuals, on the other hand, show a tendency to group about the mean. This demonstrates the relative uniformity in reproductive capacity of the parthenogenetically produced individuals in comparison with the variation among the sexually produced individuals.

In an effort further to demonstrate the genetic nature of the variations shown by the individuals hatched from sexual eggs, a few parthenogenetically produced young of some of them were cultured simultaneously as their mothers had been. Although the study of these individuals showed that environmental factors had influenced some of the differences previously noted, these latter observations for the most part provided confirmation of the differences noted between the different hatched individuals.

As referred to above, the other line, Banta's Line 1705, with the short parthenogenetic history (42 parthenogenetic generations) was studied as Line 1012 had been. Sixty females hatched from sexual eggs produced by inbreeding Line 1705 were compared with 60 parthenogenetically produced females from the same line. Unfortunately the occurrence of adverse environmental conditions terminated the experiment.

Since it is not feasible to extend this time-consuming work at present, it seems best to report the results suggested by these experiments. As far as studied, the sexually produced individuals from the line with the short parthenogenetic history showed less diversity among themselves than did those derived by inbreeding Line 1012—the line with the very long parthenogenetic history. Further, the sexually produced individuals varied little from the parthenogenetically produced females of Strain 1705, thus indicating that in this line with a short parthenogenetic history few, if any, recessive mutations had accumulated which might show up in sexually produced offspring.

These observations suggest that recessive mutations having unfavorable physiological effects accumulate in *Moina macrocopa* (as previously shown in *Daphnia longispina*) during parthenogenesis, and that the longer the parthenogenetic history of a given line, the greater the number of physiological mutations apparently revealed by the sexually produced offspring of that line.†

† The writers sincerely appreciate Dr. A. M. Banta's suggestion of the problem as well as his active interest in the work itself and in the preparation of the manuscript.

5990

Effect of a Liver Extract on Blood Sugar and Respiratory Quotient in Diabetes.*

BYRON D. BOWEN AND GRACE E. SLY.

From the Buffalo General Hospital.

The effect of whole liver and various liver extracts on the blood and urine sugar of diabetic patients has been studied by Blotner and Murphy^{1,2}. It appeared to them that 180 gm. of raw liver was equal to the action of about 10 units of insulin. The use of liver extracts (480 gm. of liver) suggested very definite blood sugar lowering properties. The average drop of the blood sugar in the control group of patients in 8 hours was 32 mg., of those receiving liver extract, 80 mg., and those receiving insulin, 10 units, 68 mg. DePencier, Soskin and Best³ made a rather extensive study of the blood sugar and sugar excretion of depancreatized dogs after the ingestion of raw liver. They did not observe that liver had any favorable effect. Gutmann and Horwitz⁴ using whole liver on diabetic patients believed that liver did exert a slightly beneficial action on the diabetes. Habs⁵ observed no essential difference in the blood sugar curves of diabetic patients after cooked liver or raw chopped beef but he did find a higher alimentary rise of the blood sugar after the ingestion of raw liver. He attributed this to the high carbohydrate content of raw liver. Bertram, Horwitz and Wahncau⁶ concluded that liver and liver extracts had no influence on the blood sugar or glucose excretion. These same workers⁷, however, later reported that when insulin was added to the aqueous liver extract and given by mouth 1½ hours before glucose, a definite decline in the blood sugar occurred, but that this action was not obtained if given with a meal. They thought that the liver extract protected the insulin from destruction by the stomach contents and that the insulin was absorbed from the stomach.

* This work was made possible by the gift of a metabolism laboratory from Mrs. William H. Crosby. The liver extract used was made by the Lederle Company and kindly sent to us by Dr. William P. Murphy.

¹ Blotner, H., and Murphy, W. P., *J. Am. Med. Assn.*, 1929, **92**, 1332.

² Blotner, H., and Murphy, W. P., *J. Am. Med. Assn.*, 1930, **94**, 1811.

³ DePencier, M. T., Soskin, S., and Best, C. H., *Am. J. Phys.*, 1930, **94**, 548.

⁴ Gutmann, K., and Horwitz, S., *Klin. Wchnschr.*, 1931, **10**, 591.

⁵ Habs, H., *Klin. Wchnschr.*, 1931, **10**, 935.

⁶ Bertram, F., Horwitz, S., and Wahncau, E., *Klin. Wchnschr.*, 1931, **10**, 1162.

⁷ Bertram, F., Horwitz, S., and Wahncau, E., *Klin. Wchnschr.*, 1931, **10**, 1214.

A MILD DIABETIC PATIENT, AGE 68.

TABLE I.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:27	5.085	0.83		33.91
9:55	4.971	0.77	111	33.32
10:12	50 gm. glucose and 100 cc. lemon juice			
10:56	5.787	0.88	242	35.73
11:48	5.525	0.87	244	34.49
12:47	5.412	0.85	194	34.02

TABLE II.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:26	4.869	0.81		34.09
9:43	4.962	0.77	125	33.35
9:55	20 units insulin			
10:25	50 gm. glucose and 100 cc. lemon juice			
11:18	5.905	0.97	194	36.08
12:33	6.018	0.94	118	34.31
1:32	5.552	0.96	100	30.42

TABLE III.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:34	5.445	0.83		36.22
9:48	4.986	0.77	118	33.09
10:05	Extract—1600 gm. liver			
10:30	50 gm. glucose and 100 cc. lemon juice			
11:13	6.112	0.84	251	38.11
11:56	5.959	0.84		37.36
1:02	5.827	0.88	221	36.30
2:07	5.501	0.86	161	33.43

Discussion. Case 1. The maximum respiratory quotient after glucose alone was 0.88 (Table I). The response to insulin was typical (Table II). The liver extract (1600 gm.) given one-half hour before glucose brought about no change in the blood sugar curve and the respiratory quotient was not raised (Table III).

Case 2. The respiratory quotient after glucose alone rose to 0.84 (Table IV). When liver extract (2400 gm.) was given one-half hour before the glucose, the highest quotient was 0.86. The blood sugar after the liver extract failed to have the usual alimentary rise with its compensatory fall (Table V). The presence of a large amount of liver extract in the stomach and small bowel might have hindered the absorption of glucose. There was no essential change in the glucose excretion when liver extract was given before the glucose.

A SEVERE DIABETIC PATIENT, AGE 24.

TABLE IV.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:24	4.113	0.78		36.28
9:39	4.071	0.73	352	37.46
9:55	50 gm. glucose and 100 cc. lemon juice			
11:01	4.776	0.84	460	38.33
12:25	4.315	0.80	450	34.63
1:58	3.856	0.74	284	34.28
Glucose excretion after glucose 28.8 gm.				

TABLE V.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:14	4.069	0.76		35.19
9:29	4.012	0.75	316	35.31
9:45	Extract—2400 gm. liver			
10:15	50 gm. glucose and 100 cc. lemon juice			
11:25	4.381	0.86	345	34.83
1:03	4.086	0.78	286	32.50
2:57	4.245	0.76	316	35.56
Glucose excretion after glucose 25.7 gm.				

TABLE VI.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:18	4.431	0.87		33.66
9:33	4.222	0.82	330	33.44
10:00	200 gm. liver			
11:07	4.413	0.80	292	37.00
1:04	4.571	0.81	276	38.11
2:54	4.507	0.85	224	34.94

TABLE VII. Normal Subject.

Time	Ventilation	R.Q.	Blood Sugar Mg. per 100 cc.	Calories/ Sq. meter/ Hour
9:12	4.294	0.81		31.91
9:39	4.272	0.79	106	32.94
10:10	200 gm. liver			
10:47	4.594	0.83	102	31.39
11:10	50 gm. glucose and 100 cc. lemon juice			
11:59	5.972	1.00		36.61
1:11	5.701	1.00	93	35.36

The effect of 200 gm. of broiled liver given to a severe diabetic patient at a time when glycosuria without acidosis was present and to a normal individual three-quarters of an hour before the ingestion of 50 gm. of glucose and 100 cc. of lemon juice: This experi-

ment was done in order to be certain that the liver itself, because of its protein and fat content, did not depress the quotient. Since the quotient in the diabetic, while having glycosuria, did not rise higher than 0.85 but did reach unity in a normal individual, it seems probable that the feeding of liver had no action on the metabolism of glucose in the diabetic.

Conclusion. The effect of liver extract on the respiratory quotient and the blood and urine sugar was studied in 2 diabetic patients. The effect of whole liver was studied in one patient. There was no evidence that either had a favorable influence on the diabetes.

5991

Electromotive Effect of Concentration of Tissue Membranes as the Result of HCl Formation.

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From the University of Louisville School of Medicine.

The large variability of certain biological potential differences with concentration¹ indicates that tissue membranes resemble metallic electrodes to some extent. This resemblance may lead to a better understanding of the nature of the electrical polarization in tissues as the cause of their irritability. Every attempt should be made, therefore, to understand the details of the physical nature of this important electrical property of tissues.

Beutner found that such a large electromotive effect of concentration can be imitated by a few water immiscible fluids, such as salicylic aldehyde.² The evidence points to *water immiscibility* and a certain *base binding power* as the essential properties for the production of a large electromotive variability with concentration. Michaelis found that collodion films also show such an effect.³ This confirms the theory, since collodion, a nitro-cellulose, has a marked base binding power, as will be demonstrated in a subsequent publication.

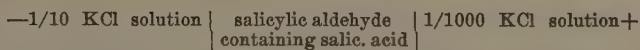
Electromotive measurements on a variety of substances and mixtures have been performed, as well as determinations of salt distribution, with the conclusion that a chemical reaction occurring at the phase boundary is the cause of the large electromotive effect of

¹ *Biochem. Z.*, 1912, **41**, 1.

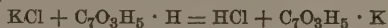
² *Am. J. Physiol.*, 1913, **31**, 443.

³ Michaelis, L., *Biochem. Z.*, 1925, **161**, 47.

concentration.⁴ For instance, the electromotive force of a system like



equals about $\frac{1}{10}$ volt. The following reaction is supposed to occur at either of the phase boundaries:



The potassium salicylate formed by this reaction accumulates in the non-aqueous solvent, salicylic aldehyde, independent to some extent of the concentration. Salicylic aldehyde in this way functions like a potassium electrode.

The objection has been raised that an entirely impossible chemical reaction is assumed. The seeming impossibility appears even more striking if we apply the same explanation to systems containing a mixture of fatty acid and cresol as central conductors. Since these systems exhibit likewise a large effect of concentration,⁴ one is led to assume that oleic acid drives the free HCl from NaCl in order to form sodium oleate. It is known, however, that oleic acid is one of the weakest acids, while HCl is about the strongest, hence HCl usually liberates the fatty acid from soap. A reaction in the opposite direction seems to be an impossibility.

And yet, such a reaction in the opposite sense actually occurs if certain non-aqueous solvents are added, even though it is impossible in aqueous solutions. For instance, in the presence of olive oil, as solvent, oleic acid actually liberates HCl from NaCl (G. H. A. Clowes⁵). Olive oil plus oleic acid would probably show an effect of concentration but on account of the very high resistance of olive oil the effect can not be demonstrated. We, therefore, used a mixture of 90% cresol plus 10% oleic acid, which mixture has a considerable effect of concentration.⁴ This mixture was suspended in water or in aqueous KCl solutions of varying concentration and NaOH added until phenol red turned red; during this titration, the fluid with the oily mixture was shaken intensively. Much more NaOH was needed when more KCl was present. Oleic acid suspended in water requires also some sodium hydroxide to turn phenol pink but if suspended in KCl solution more NaOH is needed.

In another experiment, NaOH was added to a suspension in water of cresol *without oleic acid*. In this case, *the same amount of NaOH was needed to turn phenol red pink, no matter whether KCl was added or not*. This should be expected according to our theory,

⁴ Beutner, R., *Entstehung elektr. Stroeme in Geweben*, Stuttgart, 1920.

⁵ Clowes, G. H. A., *J. Phys. Chem.*, 1916, **20**, 407.

since pure cresol has a slight effect of concentration only or none at all.

It seemed desirable to perform more reliable quantitative experiments. Various samples of the cresol oleic acid mixture, 20 cc. each, were shaken for 2 hours either with 20 cc. of water or with 20 cc. of varying KCl solutions, in the same shaking machine. The aqueous fluid of each sample was then separated from the cresol mixture and cleared by filtration. In each case 10 cc. of this fluid was mixed with 3.0 cc. of a 1/10 molecular NaOH solution and titrated back to the neutral point with phenolphthalein as indicator.

In the sample shaken with water an equivalent amount of HCl was needed to reach the turning point of the indicator, showing that no acid had been formed. In the sample shaken with KCl solution less HCl was needed to titrate back the added NaOH, indicating the formation of acid. From the excess of NaOH the following quantities of acid were calculated per 10 cc. in the sample shaken

with 1/10 molecular KCl : 0.3 cc. 1/10 molecular HCl
" 1/50 molecular KCl : 0.18 cc. 1/10 molecular HCl
" 1/250 molecular KCl : 0.11 cc. 1/10 molecular HCl

(These figures represent the averages of two well-agreeing experiments.)

Control experiments have been performed to show that this difference cannot be due to a "salt error".

Conclusions. The experiments show that water soluble acid, manifestly HCl, is formed from KCl and fatty acid, whenever an electromotive effect of concentration arises at the phase boundary. It seems likely that a reaction of the kind described is also a factor in the formation of free HCl in the glands of the stomach.

The theory of phase boundary potentials, referred to above, cannot be set forth in detail in this preliminary communication.

5992

In the Rhesus Monkey Ovulation is Spontaneous.

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In most mammals ovulation is a spontaneous event, but as first pointed out by Heape in some species, including the rabbit, the European ferret (*Mustelus putorius*), and the domestic cat, ova are

discharged from the ovary only after copulation. Such observations as these make it necessary to know the exact facts concerning each species which is used for experimental work. The common Indian monkey, *Pithecus (Macacus) rhesus*, for example is being studied extensively and used for experiments in the physiology of reproduction, but thus far the ripening and mature Graafian follicle has never been described, and no specific statement has appeared as to whether or not ovulation is spontaneous.

It is true that in the group reported by me in 1923, ovulation was shown to have occurred in females which were kept in a group with no males present, but this is not proof of strictly spontaneous ovulation because we know that ovulation may take place in rabbits kept in a common cage with other females without the presence of males. In such cases the stimulus to ovulation is provided by homosexual pseudo-copulation.

The erection in the summer of 1928 of new quarters for my colony, by aid of a fluid research fund granted to this school by the Rockefeller Foundation, permitted a direct experiment on this question.

On November 20, 1929, four fully mature female Rhesus monkeys were placed in separate cages. The animals were placed in isolation in a series of cages separated from each other by heavy wire netting. They could see each other and could touch each other by reaching their arms or legs through the meshes of the cages, but no other contact could take place. No sexual activity was observed during the experiment. We have never seen sexual contact between female monkeys by manual or pedal manipulations, homosexual activity always taking the form of pseudo-copulation. For this reason it is reasonably certain that in the experiment no sexual stimulation of other than psychic nature could have occurred. No male animals were visible from the cages in which these 4 females were housed. The animals were kept in isolation through the early winter. One of them was examined by laparotomy under ether on January 24, 1930; the others on January 27, February 14, and March 1. When explored all of them showed recent corpora lutea in their ovaries, thus indicating the occurrence of spontaneous ovulation.

5993

Effects Produced by Intravenous and Intraperitoneal Injections of Urine of Pregnancy in Immature Female Rabbits.*

J. M. WOLFE AND EUGENE T. ELLISON. (Introduced by R. S. Cunningham.)

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Nashville, Tennessee.*

Intraperitoneal injection of urine of pregnancy into mature female rabbits results in a direct luteinization of the unruptured follicles, while urine administered intravenously usually results in ovulation (Friedman¹).

We have carried out a series of experiments in which we have studied the difference in the results obtained by these 2 modes of injection of urine of pregnancy in 18 immature female rabbits. The animals were divided into 2 groups; one group received urine of pregnancy intraperitoneally, while their litter-mate sisters received an equal amount of the same specimen intravenously. The animals were injected daily for 3 to 6 days. When an animal from one group was autopsied, its litter-mate control was likewise killed. In all cases we have studied serial sections of the ovaries.

In animals whose ovaries contained no mature follicles, but did contain smaller follicles with a follicular cavity, intravenous injection resulted in extreme growth of these follicles, which at autopsy appeared cystic. They usually contained some blood but never as much as was found in the animals which had been given urine intraperitoneally. There was practically no luteinization in these large follicles. In most cases the granulosa was not present. The *theca interna* was somewhat compressed in places, in others the cells showed a marked hypertrophy. There was no change in the very small follicles. Often the ovaries in these animals contained several extremely large follicles at one pole, while the remainder of the ovary was infantile. We believe that urine injected intravenously acts only on follicles which have a well developed follicular cavity, but never causes a stimulation of the smaller and more primitive follicles.

Urine injected intraperitoneally had a very different effect. The ovaries of these animals contained a large number of small blood follicles. None of these approached in size the large follicles obtained when the urine was injected intravenously, but they were

* This work has been aided by a grant from the National Research Council Committee for Research in Problems of Sex.

¹ Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

much more numerous. There was a marked direct luteinization of most of the follicles, and they all contained varying amounts of blood. The intensity of the luteinization varied with the amount of the blood present. For example, when a greater amount of blood was present on one side of the cavity, almost invariably there was also a greater amount of lutein tissue on that side. When there was only a small amount of blood in the follicle, it was almost invariably in or near the cumulus. This suggests that the first rupture of capillaries usually occurs at this position. This observation is based on the study of serial sections of more than 100 follicles in which only a small amount of blood was present and which showed an early stage of luteinization. In the animals which received intraperitoneal injections, the smallest follicles, which contained luteinized cells and free blood in the cavity, averaged 1.41 mm. in diameter, while the largest follicles, which did not contain any blood and did not show any evidence of luteinization, averaged 1.01 mm. in diameter. Luteinization has always been accompanied by free blood in the cavity of the follicle, except where the follicle has been completely luteinized.

5994

Small Doses of Anesthetics.

CHAPMAN REYNOLDS.

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Milwaukee, Wisconsin.*

It was previously noted that, while ordinary concentrations of sodium amytal diminished or abolished tone and activity of isolated intestine and uterus, minute doses seemed to be pressor.¹ Is the same "reversal" effect to be found in whole intact animals? Picrotoxin causes convulsions in mice. The convulsions may be stopped or prevented by a proper dose of anesthetic. Indeed a method for biological assay of the hypnotics has been based on this antagonism,² and picrotoxin has been suggested as an antidote for barbiturate poisoning.³ Thus it seems that wherever the points

¹ Reynolds, C., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 656.

² Wieland, H., and Pulewka, P., *Arch. Exp. Path. u. Pharm.*, 1927, **120**, 174 and 186.

³ Maloney, A. H., Fitch, R. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1931, **41**, 465.

of action of these 2 types of poisons may be, they are sufficiently the same to exhibit a mutually antagonistic effect. Knowing that a convulsant dose of picrotoxin would be "neutralized" by a sufficient amount of anesthetic, would a sub-convulsant dose of picrotoxin become convulsant in the presence of a small, or excitatory, or stimulant dose of anesthetic?

The minimal convulsant dose of picrotoxin was determined in about 60 mice to be between 2.75 and 3.0 mg. per kg.; 2.75 mg. per kg. did not cause convulsions. Solutions of sodium pentobarbital (sodium ethyl-methyl-butyl barbiturate) and sodium amytal (sodium iso-amyl-ethyl barbiturate) were used interchangeably in equivalent doses to represent the anesthetics, since ether, alcohol or chloroform would have introduced the complicating factor of peritoneal irritation. In each experiment 3 mice were given a minute dose of a barbiturate intraperitoneally and a fourth mouse (control) approximately the same quantity of normal saline. Ten to 15 minutes later each animal received a subconvulsant dose of picrotoxin, and the group were then allowed to run freely about a table without handling or disturbance and watched for convulsions. A mouse going into unmistakable convulsions was recorded as +, otherwise, —. A typical protocol follows:

Mouse	Wt.	Barbiturate (fraction of MAD)	Time inj.	Picrotoxin (mg. per kg.)	Time inj.	Result
RA	18.2	0	1:15	2.75	1:30	—
RP	19.4	1/1000	1:17	2.75	1:32	+ 1:55
BA	19.2	1/1000	1:20	2.50	1:35	+ 1:59
BP	17.5	1/1000	1:22	2.25	1:37	+ 2:10

Results are summarized in Table I.

It is seen that convulsions are induced by non-convulsant doses of picrotoxin if these have been preceded by minute amounts of barbiturate. Apparently convulsions do not occur, however, if too little picrotoxin is used or too little or too much (*i. e.*, approaching the depressant range) barbiturate.

In terms of Bancroft and Richter's interpretation of their interesting ultramicroscopic observations of the effect of anesthetics on cell colloids,⁴ one might say that the cells of the nervous system are made more irritable by the minute amounts of barbiturates here used; but the hypothesis of a "decreased stability" state of colloids is as yet too untested to be offered as more than a tentative explanation of the herein reported phenomena.

⁴ Bancroft, W. D., and Richter, G. H., *J. Phys. Chem.*, 1931, **35**, 215.

TABLE I.

Picrotoxin (mg. per kg.)	Preceded by Barbiturate (fraction of MAD)	CONVULSIONS (Number of trials)	
		+	-
3.00	0	8	0
2.75	0	0	32
2.75	1/10	4	12
2.75	1/50	6	2
2.75	1/100	16	0
2.75	1/500	8	0
2.75	1/1000	12	0
2.75	1/2000	6	0
2.75	1/3000	2	8
2.75	1/5000	0	12
2.5	1/10	0	8
2.5	1/50	6	4
2.5	1/100	12	1
2.5	1/500	16	0
2.5	1/1000	12	0
2.5	1/2000	12	2
2.5	1/3000	0	8
2.25	1/100	4	0
2.25	1/500	6	0
2.25	1/1000	4	2
2.25	1/2000	0	10
2.0	1/100	0	6
2.0	1/1000	0	6

5995

Glucose Tolerance in Lipoid Nephrosis.

RALPH H. MAJOR AND C. J. WEBER. (Introduced by E. A. Doisy.)

From the Department of Internal Medicine, University of Kansas School of Medicine.

The behavior of patients with lipoid nephrosis to the glucose tolerance test has apparently not been extensively studied. Linder, Hill and Van Slyke¹ in their studies of carbohydrate metabolism in chronic nephritis studied 2 cases but found nothing abnormal in their response to the glucose tolerance test. These observers employed for their tests cutaneous blood obtained by a clean deep prick and made their blood determinations by the method of Hagedorn and Jensen.

We have recently studied the response of 4 patients with typical lipoid nephrosis to the glucose tolerance test and have obtained atypical curves in all these patients. Venous blood was employed

¹ Linder, G. C., Hill, A., and Van Slyke, D. D., *J. Clin. Invest.*, 1925, **1**, 247.

for the blood sugar determinations by the method of Folin and Wu. None of these patients showed glycosuria and for this reason only the blood sugar determinations will be reported.

Fig. 1 shows the results of glucose tolerance carried out on the patients employing 1.5 gm. and 3 gm. glucose per kilo body weight. In none of these tests does the blood sugar rise above 0.10 gm. per

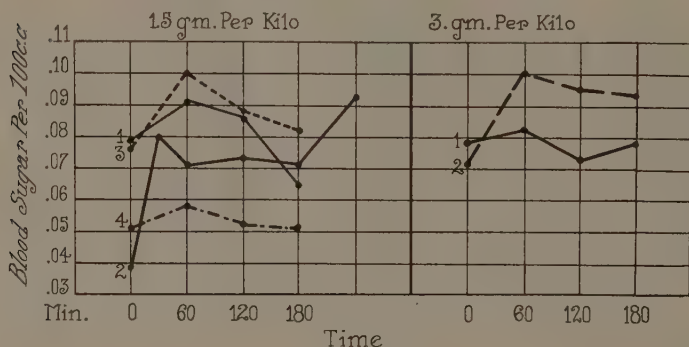


FIG. 1.

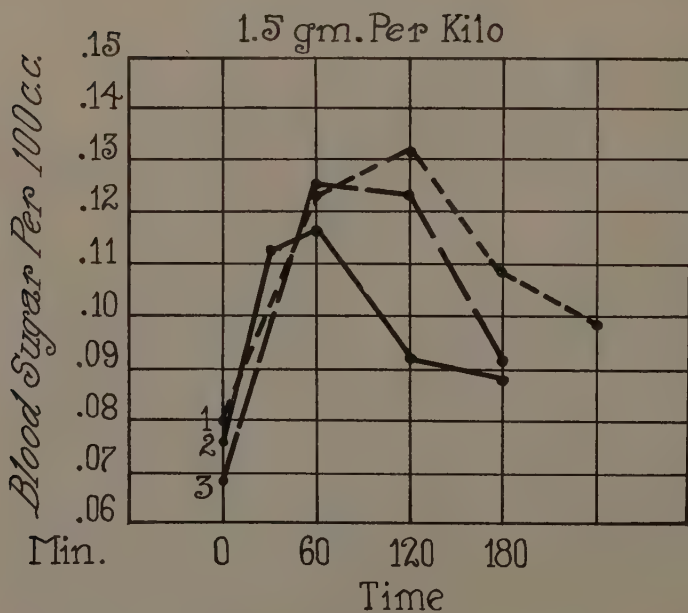


FIG. 2.

100 cc. and we do not see the characteristic sharp peak with rapid fall characteristic of normal individuals.

These patients were placed upon a high carbohydrate diet and treated with diuretics. Following marked clinical improvement, the tests when repeated showed the results charted in Fig. 2. These curves are identical with those of normal individuals.

We have occasionally obtained curves similar to those shown in Fig. 1 in patients with myxedema, mild hyperthyroidism and pituitary disease. Hamman and Hirschman² describe a similar curve in a normal control. The presence of such curves in all 4 of these patients with nephrosis is worthy of further study. It is difficult to explain these curves on the basis of a thyroid deficiency. At the time the curve on patient I, shown in Fig. 1, was obtained, the patient had been taking thyroid extract for 6 weeks and had a normal metabolic rate. The curve on patient III, shown in Fig. 2, which is a normal curve, was obtained on a patient whose metabolic rate on the day of the test was —21 and who had received no thyroid extract.

These atypical curves suggest at once the possible factor of poor absorption from the gastro-intestinal tract. In patients I and II the oral administration of 15 gm. of urea simultaneously with the glucose produced a sharp rise in the blood urea of these patients, proving that the urea was promptly absorbed. In patient IV two glucose tolerance tests carried out by the intravenous method employing 0.2 gm. per kilo and 0.3 gm. per kilo showed blood sugar curves almost identical with the curve of this patient shown in Fig. 1. In patient I an intravenous glucose tolerance test carried out a few days after the test shown in Fig. 2 was performed showed a normal blood sugar curve.

² Hamman, Louis, and Hirschman, I. I., *Arch. Int. Med.*, 1917, **20**, 761.

5996

pH Changes Accompanying Filtration Through Cellophane Membranes.

FRANK URBAN AND H. L. WHITE.

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Washington University School of Medicine.*

Previous studies¹ led us to expect pH changes on filtration of buffers through cellophane membranes. We present a preliminary report of data which confirm our expectations.

We used phosphate buffers of pH 7.0 and 7.2, having phosphate concentrations of 0.005 M and 0.01 M. They were prepared by the addition of CO₂-free NaOH to twice recrystallized NaH₂PO₄·H₂O. The 0.5 M stock solution was diluted with conductance water. Prior to filtration the cellophane 600 plain membranes were evacuated at 20 mm. Hg. and the buffer solution added. After repeated washing and soaking for several days the unsupported membrane was placed between pyrex cylinders of 2 cm. diameter and filtration carried out at 38 cm. Hg. The upper cylinder, into which the filtrate passed, communicated with a 125 cc. Erlenmeyer flask containing some of the buffer. Concentration of the filtrate by evaporation was thus prevented. The pH measurements were carried out according to the micro method of Bodine and Fink,² quinhydrone being used. A 25°C. ± 0.01° thermostat and type K Leeds & Northrup potentiometer were used. pH determinations of the original buffer and of the filtrate were carried out simultaneously. As we are here interested only in pH differences, the quinhydrone salt error was not evaluated. About 30 filtrates were examined with 2 or more pH determinations on each filtrate. The maximum fluctuation on duplicate determinations was 0.015 pH. The filtrate of the 0.005 M pH 7.2 phosphate buffer showed a maximum change toward the acid side after 3 hours of 0.46 pH and an average of 0.24 after from 2 to 15 hours. The filtrates of 0.01 M pH 7.2 and pH 7.0 gave maximum changes after 1 hour of 0.26 and 0.28 pH, respectively, and averages of 0.17 and 0.19 after from 1 to 3 hours, always toward the acid side. Shorter filtration periods were not investigated. Two membranes were used throughout. After approximately 130 hours of discontinuous filtration these membranes still showed the same effect. In order to evaluate the combined

¹ Bishop, G. H., Urban, F., and White, H. L., *J. Phys. Chem.*, 1931, **35**, 137.

² Bodine, J. H., and Fink, D. E., *J. Gen. Physiol.*, 1925, **7**, 735.

influence of atmospheric CO_2 and membrane a few drops of buffer solution were left in contact with squares of cellophane treated as the filters had been. These were placed in small Petri dishes and left from 2 to 17 hours. The maximum changes were 0.02 pH, barely beyond the limits of the method. If small amounts of acid had been present in the membranes it seems reasonable that they would have disappeared after prolonged soaking. The control results substantiate this opinion. Cellophane 600 plain is not a molecular sieve; a P.D. of only a few millivolts is observed across it with KCl solutions with a concentration ratio of ten to one.

5997

Effect of Ions on Filtration Rates Through Cellophane Membranes.*

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Washington University School of Medicine.*

When distilled water is filtered through cellophane membranes which have been evacuated prior to several days' soaking in water, it is found that the rate of filtration usually decreases during the first hour, finally reaching an essentially constant value. We predicted that it should be possible to raise or lower the value of this final "steady" rate of filtration by adding varying amounts of ThCl_4 to the water. The preliminary results given below show that the steady rate of filtration is affected by ThCl_4 .

The membrane was placed between glass cylinders and supported by a coarse alundum disc. Filtration was carried out at 10 cm. Hg. The rate of filtration was determined by following through a microscope equipped with a micrometer eye-piece the displacement of the meniscus in a capillary. A solution of 10^{-3} gms. ThCl_4 per liter gave a 25% increase in the rate of filtration over that of distilled water. Solutions of 1 gm. and of 10 gm. ThCl_4 per liter showed a decrease in the rate of filtration of 29% (average of 5 determinations) and 35% (average of 3 determinations), respectively. A solution of 10^{-2} gm. ThCl_4 per liter showed no large effect. A hypothesis concerning the mechanism of these processes, together with an extension of experimental results, will appear in a subsequent communication.

* This work was aided by a grant from the Science Research Fund of Washington University.

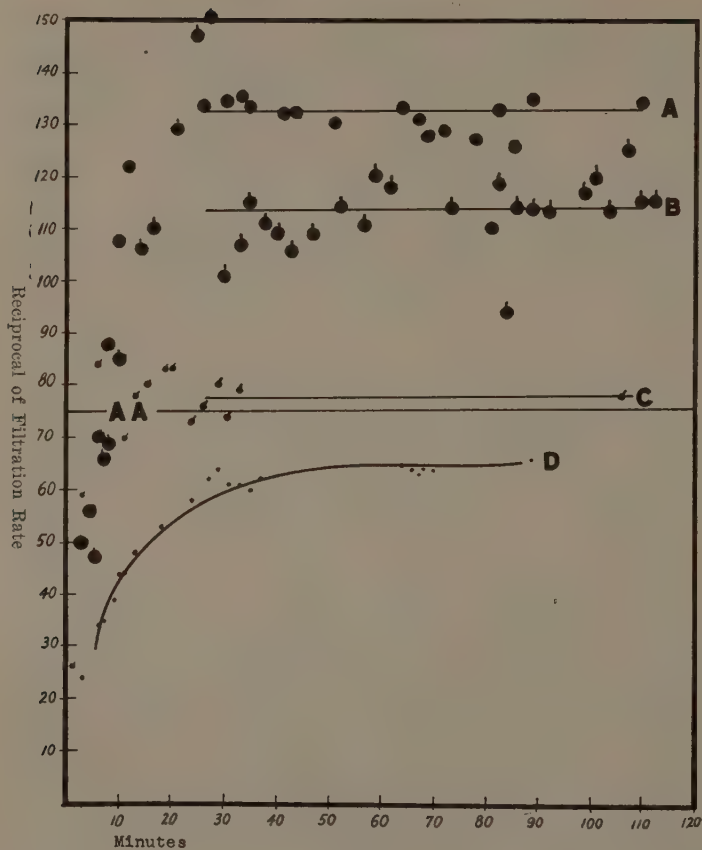


FIG. 1.

AA is curve for water, A for 10 gm. ThCl₄/liter, B for 1 gm. ThCl₄/liter, C for 10⁻² gm. ThCl₄/liter, and D for 10⁻³ gm. ThCl₄/liter. Ordinates reciprocal of filtration rate, abscissas in minutes.

5998

Significance of Creatine in Progressive Muscular Dystrophy, and Treatment of this Disease with Glycin.

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From the Physiologisch-Chemisches Institut, Leipzig.

Ever since Duchenne (de Boulogne)¹ made his classical studies of the progressive muscular atrophies the pathogenesis has been a subject of much contention. The view held by Duchenne¹ that the disease is primary in the muscles is supported by the clinical and pathological findings of many able workers,^{2,3} but despite this, practically all methods of treatment have been based on the theory that the condition is secondary to some disturbance in the innervation.^{4,5}

Patients with this disease show a creatinuria even when maintained on a creatine-free diet, and in contradistinction to the normal subject excrete ingested creatine almost quantitatively (65-100%). This inability to retain ingested creatine is in proportion to the severity to the disease.

In our investigations on the origin of creatine,⁶ many substances that could be considered biologically related to creatine were studied. We were able to confirm the findings of Gibson and Martin⁷ who administered gelatin, and of Brand, Harris, Sandberg, and Ringer⁸ who were the first to recognize the relationship of glycin, that the ingestion of this amino acid in these cases is followed by a large increase in the creatinuria. This significant relationship between glycin, a substance which the normal subject can readily synthesize,

* Fellow in Medicine, National Research Council.

¹ Duchenne, G. B. (de Boulogne), *De l'électrisation localisée*, 2nd Ed., Paris, 1861.

² Erb, W., *Arch. f. klin. Med.*, 1884, **34**, 467; *Zeitschr. f. Nervenheilkunde*, 1890, **1**, 1.

³ Friedreich, N., *Ueber progressive Muskelatrophie*, Berlin, 1873.

⁴ Kuré Ken, *Die vierfache Muskelinnervation einschliesslich der Pathogenese und Therapie der progressiven Muskeldystrophie*, Urban u. Schwarzenberg, Berlin, 1931.

⁵ G. de N. Hough Tr., *J. Bone and Joint Surg.*, 1931, **13**, 669.

⁶ To appear shortly in Hoppe-Seyler *Z. f. physiol. Chem.*

⁷ Gibson, P. B., and Martin, F. T., *J. Biol. Chem.*, 1921, **49**, 319.

⁸ Brand, E., Harris, M. M., Sandberg, M., and Ringer, A. L., *Am. J. Physiol.*, 1929, **90**, 296. Brand, E., Harris, M. M., Sandberg, M., and Lasker, M., *J. Biol. Chem.*, 1930, **87**, lix. Brand, E., Harris, M. M., *J. Biol. Chem.*, 1931, **92**, Proc. 1 ix.

and creatine, which has been demonstrated to play an important rôle in muscle function, suggested that the prolonged administration of this amino acid might have an influence on the clinical and chemical course of this disease. We have studied 6 patients during 8 experimental periods each of a few months' duration. Three of these patients had the clinical picture known as progressive muscular dystrophy, and 3 the symptom complex designated as pseudo-hypertrophic muscular atrophy. The daily ingestion of 5 gm. of glycine was followed by a definite rise in the creatinuria; 15-20 gm. increased the daily excretion of creatine 300-500 mg., the more advanced cases excreting the larger amounts. The percentage increase naturally depends upon the height of the control level. In one case the increase was 100% (500 mg.); in others where the previous creatinuria level was low the smaller increase (300 mg.) represented a percentage increase of 1000%.

After a period of some weeks (depending on how advanced the case is) the creatinuria begins to decrease despite the continuance of glycine until it falls to the former control level. In some cases this requires only 2 to 3 weeks; in one advanced case even after 8 weeks the previous level had not been reached. In one patient the creatinuria fell to a value below that observed before glycine was given. Coincident with the decrease in the creatinuria there is a rise in the creatinine output, and an improvement in the patient's ability to hold ingested creatine. These changes in the metabolism disappear in the course of a few weeks after the cessation of glycine ingestion, and return if the administration is again instituted.

While these changes in the metabolism are taking place, the condition of the patient improves in a remarkable manner. The first symptom manifested in half of our patients was a curious feeling in the muscles which they describe as a "crawling, rumbling" sensation, which in one case was so severe that sleep was definitely interfered with. In these patients muscle groups affected were those whose function was most impaired. One patient who was given glycine during 2 periods 6 months apart experienced the sensation in the same muscle groups both times. This curious feeling makes its appearance some days before the creatinuria begins to fall, and disappears after further administration of glycine in 3 to 14 days. One patient had such muscle sensations on admission, but after glycine administration they completely disappeared for the first time in over a year, and did not return.

The next change in the clinical course is the disappearance of the fatigue that frequently constitutes a prominent and distressing symp-

tom in this disease. The muscles that had previously felt tired and heavy now feel refreshed and lighter, and the patients often desire to make muscular movements they have not been able to make for a long time. Gradually the function of certain muscle groups so improves that activities can be performed that had been impossible for years (stair climbing, arising from the floor unaided, bicycle riding). The degree of improvement differs not only in different patients, but in different muscles of the same patient. The time required for such improvement varies widely. One patient showed a remarkable improvement in 3 weeks; in another no definite increase in muscle function could be demonstrated at the end of 9 weeks, although there was much subjective improvement.

Two patients who showed considerable improvement after glycine administration were permitted to interrupt the treatment for 3 months. The improvement continued for another 3 to 4 weeks, and then gradually disappeared until their condition returned to its previous status. The changes in the metabolism corresponded to these changes in the clinical condition. One of these patients has again been given glycine, and after 3 weeks shows the same remarkable improvement in the same muscle groups as before.

Besides these cases we have studied, as controls, 3 patients with extensive muscular atrophy due to other causes (amyotrophic lateral sclerosis, severe chronic rheumatism of the joints, congenital idiocy). These patients also showed a creatinuria on a creatine-free diet, and could retain only about 50% of ingested creatine. The administration of glycine was followed by no increase in the creatinuria. It is suggested that after further studies of this kind the reaction to glycine might be of value in the differential diagnosis of the muscular atrophies. During 5 weeks of glycine administration (15 gm. daily) the patients felt in no way improved, and had none of the symptoms experienced by our other patients. At the end of the period no change in the metabolism could be demonstrated, and the inability to retain ingested creatine had not improved.

These findings, in our opinion, indicate that the improvement obtained in our cases of progressive muscular dystrophy and pseudohypertrophic muscular atrophy is not due to any temporary irritative action of this amino acid, but that glycine has an important function in muscle physiology, and a significant rôle in the pathogenesis and treatment of this disease.

Respiration of Isolated Nerve.*

WALTER S. ROOT. (Introduced by Frank P. Knowlton.)

*From the Department of Physiology, College of Medicine, Syracuse University,
and the Marine Biological Laboratory.*

Forty-three experiments on the rate of respiration of the lateral line nerve of the dogfish have been carried out by the tonometric method during the first 27 hours after isolation. Fenn's¹ statement that the rate of oxygen consumption of these nerves at 22.0°C. increases rapidly several hours after removal from the body was confirmed. This increase which begins 6 or more hours after isolation may reach a value nearly twice that of the initial rate of oxygen consumption at the end of 27 hours. During this time the rate of carbon dioxide production remains relatively constant. The apparent respiratory quotient, therefore, falls from 0.82 to 0.50.

Determinations of the carbon dioxide binding power of these isolated nerves indicated that this property is relatively constant during the first 12 hours. From 12 to 32 hours after isolation there is an enormous increase in the amount of carbon dioxide bound. This value becomes 500 volumes per cent at the end of 30 hours. Assuming the applicability of the Henderson-Hasselbalch equation, the pH is calculated to have increased from about 6.6 during the first 12 hours to 8.4 at 32 hours. This would indicate a liberation of approximately 16×10^{-5} molar equivalents of base.

Calculation of the true respiratory quotient gives values which increase from about 0.82 during the first 6 hours to 5.80 at the end of 24 hours.

It is possible that this high carbon dioxide production may be due to enzymatic or bacterial splitting of urea which is present in large amounts.² Experiments will be carried out to determine the effect of bacterial contamination and the source of the base liberated.

* Aided by a grant from the Hendricks Research Fund, Syracuse University.

¹ Fenn, W. O., *Am. J. Physiol.*, 1927, **80**, 327.

² MacCallum, A. B., *Proc. Roy. Soc.*, 1909, **82B**, 602.

6000

Nerve Impulses from Single Receptors in the Eye of *Limulus*.

H. K. HARTLINE AND C. H. GRAHAM.* (Introduced by D. W. Bronk.)

From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Mass.

One of us¹ has reported "small but definite electrical changes" in the optic nerve of *Limulus polyphemus* when the eye is exposed to light. The present communication continues this study with improved methods which have made it possible to record impulses in single fibres of that nerve, by means of a battery coupled amplifier and Matthews' oscillograph.

The compound lateral eye of *Limulus* is an admirable preparation for the study of photoreception. Each of the large and widely spaced ommatidia is supplied with one nerve fibre which runs directly to the central ganglion. In the eye itself there is no evidence of either ganglion cells or neural interconnections. The optic nerve is easily obtained and the eye, together with a centimeter or more of optic nerve attached, may be excised and will survive 8 hours or longer.

Action potentials from the whole nerve were studied in the young animal. When the eye is illuminated there is developed a succession of slow potential changes, superimposed on which are the typical rapid changes associated with the conduction of nerve impulses. In the whole nerve these impulses are quite irregular and resemble the responses recorded from the optic nerve of *Conger vulgaris* by Adrian and Matthews.²

A study of the discharge of impulses from a restricted number of sense cells is made possible by the circumstance that the optic nerve of *Limulus* contains little connective tissue, and hence can be separated into bundles containing only a few fibres. Moreover, in the adult animal it appears that many of the ommatidia are no longer functional, and it frequently happens that in a small bundle separated off from the whole optic nerve there is only one active fibre.

Fig. 1 is a record obtained from such a bundle. The nerve impulses demonstrated are usually large (ca. 0.6 millivolts), due in part, at least, to the fact that there is little short-circuiting material surrounding the active fibre. The regularity in the discharge of the

* National Research Fellow in the Biological Sciences.

¹ Hartline, H. K., *Am. J. Physiol.*, 1928, **83**, 466.

² Adrian, E. D., and Matthews, R., *J. Physiol.*, 1927, **63**, 378.

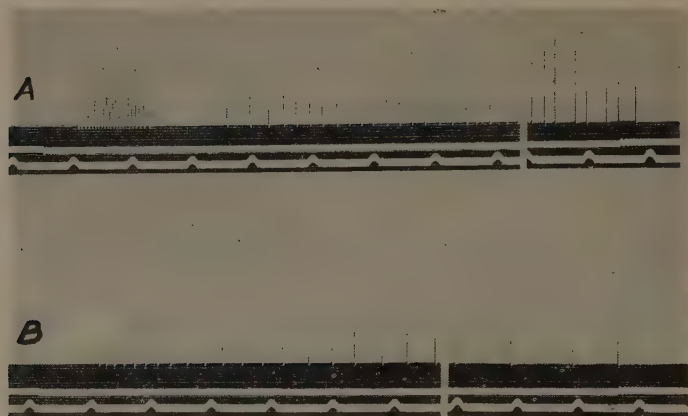


FIG. 1.

Oscillograph records of potential changes in a small bundle of fibers from the optic nerve. Leads taken from the cut end of the bundle and an uninjured point about 5 mm. distal to it. Increasing negativity of the distal lead produces a deflection of the shadow edge upwards. Time tracing (at bottom) in fifths of seconds. Deflection downwards of the signal (above time tracing) indicates the onset of illumination. Area of eye illuminated = 0.01 mm.^2 Complete dark adaptation in both records.

A. Intensity of stimulation = 1.00 (arbitrary units). The gap in the record represents an interval of 7.2 sec. B. Intensity of stimulation = 0.014 (arbitrary units). The gap in the record represents an interval of 9.0 sec. (1 arbitrary unit of intensity equals approximately 85,000 meter candles on the surface of the eye.)

impulses and their uniformity in size indicate that only one visual element is concerned. When the bundle contains more than one active fibre, the impulses belonging to each are clearly recognizable. Aside from the fact that each one forms its own definite regular sequence, the impulses in any 2 fibres can readily be distinguished by differences in the magnitude and form of the recorded response. Moreover, the photoreceptors attached to these fibres have different thresholds and discharge impulses at different rates.

The impulse discharge from these receptors resembles that found by previous investigators from other sense cells, *e. g.*, tension receptors in muscle.^{3, 4, 5} The discharge starts after a short latent period at a high frequency, falls rapidly at first, and then more slowly, tending to reach a constant value after several minutes. The impulses stop when the light is turned off. The frequency of impulses in both the initial burst and final steady discharge depends on

³ Adrian, E. D., and Zotterman, Y., *J. Physiol.*, 1926, **61**, 151.

⁴ Bronk, D. W., *J. Physiol.*, 1929, **67**, 270.

⁵ Matthews, B. H. C., *J. Physiol.*, 1931, **71**, 64.

the intensity of the stimulating light (*cf.*, Fig. 1, A and B). Dark adaptation also modifies the discharge frequency, *i. e.*, the end organ responds to a stimulating light of given intensity with an initial frequency which is greater the longer the eye has been in the dark.

The short pause following the initial burst of impulses, observable in Fig. 1A, is a characteristic response of the completely dark adapted eye to light of high intensity. This "silent period" is absent if the eye is not completely dark adapted, or if the intensity of the stimulus is low. The discharge then consists of an unbroken succession of impulses regularly decreasing in frequency (*cf.*, Fig. 1B).

6001

Time-Intensity Relations for the Stimulation of Tissue by Electric Currents.

H. A. BLAIR. (Introduced by Carl J. Wiggers.)

From the Department of Physiology, Western Reserve University Medical School, Cleveland.

Justification is felt in advancing a new analysis of the time-intensity relations for electrical stimulation as it is much simpler mathematically than any existing general analysis and therefore more useful in planning and co-relating experiments while at the same time it fits, as well or better, the experimental data of the Lapicques.

The derivations are based on the following hypotheses: (a) The rate of growth of the local excitatory process whose magnitude is designated by p is proportional to the applied voltage. (b) The tissue has a reaction toward normal at a rate proportional to p . (c) When p reaches a liminal value h excitation is accomplished.* The differential equation expressing (a) and (b) is,

$$\frac{dp}{dt} = KV - kp$$

where V is the applied voltage, K and k are constants.

For direct current a solution is,

$$kt = \log \frac{KV}{KV - kh} = \log \frac{V}{V - R}$$

when the rheobase, R , is substituted for kh . All the direct current data in Lapicque's book³ fit the equation,

* h is probably in general a function of the voltage, *e. g.*, when C is not zero with direct currents.

$$\log \frac{V}{V-R} = kt + C$$

very well, C being usually positive. With the newer data of Lapicque,^{1, 2} the fit is as good, C being positive sometimes, sometimes negative, frequently zero. The new data indicate that C depends on the type of electrodes used.

In the following examples of the agreement of the equation with Lapicque's data the values marked with asterisk were used to calculate the constants.

Helix-nerve muscle, ³ page 214. $k = 10.24$. $C = .0636$.						
t secs.	.014	.021	.036	.057	.086	∞
V obs.	*8.2	6.4	5.0	*4.0	3.5	3.1
V calc.	8.2	6.5	4.9	4.0	3.5	3.1
Frog-sartorius, fluid electrodes, ¹ page 206. $k = 292.3$. $C = 0$.						
t secs.	.001	.002	.003	.005	.010	.020
V obs.	*4.5	3.2	2.45	2.3	2.2	2.2
V calc.	4.5	3.0	2.5	2.3	2.2	2.2

The second example is typical for fluid electrodes in that $C = 0$, or is small. This was also pointed out by Lapicque.¹ He derived, at one time,⁴ an expression equivalent to the above equation using a leaky condenser model. He did not include C , so discarded the equation on account of the poor agreement with experiment.

Chronaxie is given by,

$$T = \frac{1}{k} (\log 2 - C)$$

For condenser discharges the solution is,

$$P = \frac{Kq_0r}{crk-1} \left\{ \begin{array}{l} \frac{-t}{er} \\ e^{-kt} \\ -e \end{array} \right\}$$

where q_0 , c , are the initial charge and the capacity, respectively, of the condenser, and r is the series resistance. This equation has a maximum when

$$t = \frac{er}{1 - crk} \times \log \frac{1}{crk}$$

This is the predicted *durée utile* of the discharge. The following example is from Lapicque's data:

¹ Lapicque, L., *J. Physiol.*, 1931, **73**, 189.

² Lapicque, L., *J. Physiol.*, 1931, **73**, 219.

³ Lapicque, L., *L'excitabilité en Fonction du temps*. Presses Universitaires, Paris, 1926.

⁴ Lapicque, L., *J. Physiol., Path. gen.*, 1907, **9**, 622.

Frog sciatic-gastrocnemius,⁵ page 701. $k = 298.4$.

durée obs. secs.	*.00181	*.00154	.00135	.00093	.00063
durée cal. secs.	.00181	.00154	.00147	.00091	.000602

The general equation for condenser stimulation is

$$h = K_{q0r} (\text{erk})^{\frac{\text{erk}}{1 - \text{erk}}}$$

For chronaxie,

$$T_c = -\frac{2}{k} \log 2$$

The relation of direct current chronaxie to cr is,

$$T = \frac{\log 2 - C}{2} cr$$

When $C = 0$, which is probably the ideal case,

$$T = cr \frac{\log 2}{2} = .3466 cr$$

This is close to Lapicque's mean value from experiment, 0.37 cr .

The summation of condenser stimuli gives

$$h = \frac{K_{q0r} e^{-\frac{t}{cr}}}{\text{erk} - 1} \left\{ \frac{1}{\theta} + \frac{1}{\theta} + \frac{1}{\theta^2} + \dots + \frac{1}{\theta^{n-2}} \right\}$$

where $\theta = e^{kt}$ and t is the duration of each stimulus as well as the interval between them. A similar expression derived by Lapicque from other considerations fitted the experiments of the Chauchards.⁶

For currents of linear and exponential rise solutions are easily obtained but there is no data suitable to test them.

For alternating current the solution is

$$p = \frac{KV_0}{k^2 + w^2} \left\{ k \sin wt - w \cos wt + we^{-kt} \right\}$$

when the applied voltage is $V = V_0 \sin wt$. The transient term we^{-kt} may account for the twitch observed at voltages lower than those required for tetanus. Neglecting the transient term the maxima of the equation are given by,

$$p = \frac{KV_0}{k^2 + w^2}$$

which equals h when the stimuli are adequate. When w is large compared to k ,

$$V_0 = \frac{h}{K} w$$

⁵ Lapicque, L., *Compt. rend Soc. Biol.*, 1907, **62**, 701.

⁶ Evans, C. Lovatt, *Recent Advances in Physiology* (4th ed.). 1930.

This conclusion that the applied voltage may be proportional to the frequency at high frequencies has been reached through experiment also.⁷ The second to last equation fits new experimental data using frequencies from 400 to 40,000. K and k change, however, although particular values apply to large frequency ranges.

6002

The Thoracic Window.

WALTER L. MENDENHALL. (Introduced by Sanford B. Hooker.)

From the Department of Pharmacology, Boston University School of Medicine, the Evans Memorial, and the Massachusetts Memorial Hospitals, Boston.

Direct observations of activity in the lungs and heart have always been complicated by the fact that artificial respiration has been required to keep the animal alive. The results of the observations were often vitiated by the lung movements and consequent difficulty of maintaining a focus when microscopic observations were made.

Hall¹ described a method in which the isolated lung was studied by transillumination. The method was complicated and observations were limited to a rather short survival period. Later, Wearn² developed a much simpler method in which the animal was not exposed to excessive surgery. The tip of a lower lobe of the lung was used. Illumination was secured by a light directed through the diaphragm. Observations were made through the thinned thoracic wall. Intratracheal insufflation was used. The field for observation was definitely limited to a small area in the lowest portion of the lung.

The thoracic window is another method of making direct observations not only of the lungs and heart grossly, but also microscopically. Artificial respiration is not needed. The animal (cat or rabbit) is thoroughly anesthetized. An oval section of skin is removed from the anterior aspect of the chest. The skin remaining on the thorax is separated from the underlying muscles. Artificial respiration is begun when the chest is opened. Several ribs are resected on each side at a point about midway between the sternum and vertebral column. After removal of the ribs and sternum

⁷ Asher, Leon, *Skand. Arch.*, 1923, **48**, 6.

¹ Hall, Harry L., *Am. J. Physiol.*, 1925, **72**, 446.

² Wearn, J. T., Ernestene, A. C., and Bromer, A. W., *Am. J. Physiol.*, 1928, **85**, 410.

a hole is punched through the chest wall out through the skin. A catheter is then drawn through this opening, the end resting inside the chest cavity. A window of clear photographic film is cut to fit the opening in the chest. It is made larger than the skin incision. The window is now placed over the chest opening, on the thoracic muscles, but *under* the skin. The skin is pressed down carefully and firmly on the window. By suction applied to the exposed end of the catheter, negative pressure is re-established in the new intrathoracic cavity. The catheter is closed by means of a clamp. Artificial respiration is now discontinued. The animal is capable of carrying on its own respiration for many hours with the thoracic window in place. The lungs and heart are thoroughly exposed to view.

The method is available for numerous and various observations on vascular and bronchial reactions to intravascular or intrapulmonary administrations of drugs or other agents. The heart may likewise be readily observed. The animal shows no distress. No indication of imperfect oxygenation of the blood is apparent. The window becomes sealed in place by means of the exuded tissue juices and remains air-tight, just as a similar window when placed in the abdominal cavity.³

The advantages of this method are: 1. Artificial respiration is not required, thereby eliminating obvious difficulties. 2. A large area of lungs or heart is exposed so that suitable fields for microscopic observation may be selected. 3. It may be used to study the effect of intravenous injection of drugs on heart and lungs either macroscopically or microscopically, the effects of inhalations of various agents, of nervous stimulation, or of local applications on the heart or lungs. 4. It has the possibility of clinical application in the treatment of diseased conditions in the thorax. Placing a quartz window in the chest over a diseased area for the purpose of irradiation of the area has been considered.

³ Mendenhall, Walter L., *Science*, 1931, **74**, 245.

6003

Purification of Corporin.*

H. L. FEVOLD AND F. L. HISAW.

From the Department of Zoology, University of Wisconsin.

The purification of corporin, the hormone of the corpus luteum responsible for progestational modification in the uterine mucosa, has been reported.¹ However, in recent work with this hormone we have purified the active material still further and have obtained a crystalline product which gives the characteristic physiological reaction when injected in female rabbits castrated in heat.

Method. The extraction and purification is carried out as previously described.¹ The 90% methyl alcohol solution containing the active material is evaporated and the solute taken up in 50% methyl alcohol (100 cc. per kg.) and extracted 3 times with an equal volume of low boiling petroleum ether. Only a small amount of activity is left in the 50% methyl alcohol solution, so it is discarded. The petroleum ether solution is evaporated and the solute is taken up in 15 cc. of 66% ethyl alcohol. This is chilled at 0°C. for 8-10 hours and the oily material is centrifuged off, redissolved in 66% alcohol and reprecipitated. After the second precipitation the supernatant fluids are united and evaporated to dryness and taken up in 5 cc. 50% ethyl alcohol. Any oily material which separates out is removed and discarded. Enough water is then added to make a 40% solution of alcohol and the solution is kept between 0°C. and -10°C. Crude crystalline material begins to separate out and is centrifuged off and recrystallized from 35-45% alcohol. After several crystallizations the resulting product consists, as far as can be determined microscopically, of colorless crystalline material.

The crystals are characteristically needlelike in shape if rapid crystallization has taken place. If crystallization has been allowed to proceed slowly they are what might be characterized as dagger-like plates or rods. They are readily soluble in alcohol, ether, acetone or other similar solvents but much less soluble in water. They are readily recrystallized and after several recrystallizations and washings with cold 35% alcohol still retain their activity. The smallest

* This work has been assisted by grants from the Committee for Research on Problems of Sex of the National Research Council and by the University of Wisconsin Research Funds.

¹ Fevold, H. L., Hisaw, F. L., and Leonard, S., *J. Am. Chem. Soc.*, 1932, **54**, 254.

amount of the crystalline fraction required to give the progestational reaction has not yet been determined but the yield in terms of rabbit units is low as compared with the activity of the original extract. However, the activity of the material is very high when calculated on the dry weight of the crystals, much higher than any other preparation we have thus far obtained. At present we do not know that the crystals are the pure hormone but the activity seems, at least, to be closely associated with them.

The uteri of rabbits, which show pronounced progestational modification as a result of treatment with the crystalline preparation, continue to give a strong response to pituitrin. (Details will be reported by M. A. Foster.) This is in direct contrast to the response of the uteri of pregnant and pseudopregnant rabbits or those which have received crude corporin preparations.^{2, 3, 4, 5}

6004

Effects of Administration of Cortical Adrenal Extract to the Hypophysectomized Anuran.

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Among the results of early hypophysectomy in the anuran larva is marked underdevelopment of the thyroid gland and the adrenal cortex.¹ The resulting condition of the sex-glands has not been adequately studied in amphibia although it is well known for mammals.² In attempting to analyze this fairly complex glandular situation from a functional viewpoint it seems important to determine what effects are due primarily to absence of the hypophysis and what secondarily, through impairment of the thyroid, the adrenal cortex, or possibly the gonads. The relationship of the thyroid has been rather definitely established by the following observations: (1) anterior pituitary substance to hypophysectomized tadpoles stimulates the atrophic thyroid and induces metamorphosis; (2) thyroid substance to hypophysectomized tadpoles produces metamorphosis;

¹ Knaus, H., *Arch. f. Gynäk.*, 1930, **2**, 141.

² Knaus, H., *Arch. f. Exp. Path. u. Pharm.*, 1930, **5**, **6**, 151.

³ Reynolds, S., *Am. J. Phys.*, 1931, **2**, 98.

⁴ Robson, J. M., and Ellingworth, R. E., *Quart. J. Exp. Phys.*, 21, **1**, 93.

⁵ Smith, P. E., *Am. Anat. Mem.*, 1920, **11**.

² Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

(3) pituitary substance to hypophysectomized tadpoles which also have been thyroidectomized does not cause metamorphosis.^{1,3} It is clear that the hypophysis works through the thyroid in this particular reaction.

The functional relationships of the adrenal cortex are not so well known, although it has been shown that pituitary administration restores the atrophied adrenal cortex of the hypophysectomized animal.^{1,2} Due to its diffuse nature and its intimate anatomical relation to the mesonephros, the amphibian adrenal is not suitable for experimental removal. Until the recent work of Hartman *et al.*,⁴ and of Swingle and Piffner⁵ a potent extract of the adrenal cortex was not available. A relation of the adrenal gland to sex function is suggested by numerous clinical and pathological observations.⁶ Nice and Shiffer⁷ report early opening of the vaginal canal. Corey and Britton⁸ induced precocious sexual maturity in the rat. There was hypertrophy of the hypophysis in 20-day rats. It is suggested that the cortical adrenal extract acted through the hypophysis. Connor⁹ was unable to confirm the work of Corey and Britton and found that watery extracts of the adrenal cortex contain a substance which inhibits the activity of the gonads of mature animals. Freed, Brownfield and Evans¹⁰ report degeneration of the testis following adrenalectomy in the white rat, either mature or immature.

Larvae of *Rana sylvatica* were hypophysectomized at the tail-bud stage. At different ages thereafter groups of animals were given cortical adrenal extract (Hartman) by intraperitoneal injection, using special fine hypodermic needles. Female animals treated for a period of about 2 months beginning at 5 months of age show a definite stimulation of the ovaries. Younger animals treated for periods of 2 or 3 weeks did not exhibit this effect. The ovaries of treated animals average $2\frac{1}{2}$ times the size of those from hypophysectomized untreated controls. Thirty specimens of *Rana sylvatica* were used. Of these 15 received cortical extract. Fourteen were silvery (hypo-

¹ Allen, B. M., *Quar. Rev. Biol.*, 1929, **4**, 325.

⁴ Hartman, F. A., Brownell, K. A., and Hartman, W. E., *Am. J. Physiol.*, 1930, **95**, 670.

⁵ Swingle, W. W., and Piffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153.

⁶ Barker, L. F., and Hoskins, R. G., *Endo. and Metab.*, 1922, **2**, 345.

⁷ Nice, L. E., and Shiffer, A. L., *Endocrinology*, 1931, **15**, 205.

⁸ Corey, E. L., and Britton, S. W., *Science*, 1931, **74**, 101; *Am. J. Physiol.*, 1931, **99**, 33.

⁹ Connor, C. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 131.

¹⁰ Freed, S. C., Brownfield, B., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 1.

physectomized) controls; 1 was a dark (normal) control. In addition many normal controls of other series were available for comparison.

Histologically untreated animals show ovaries with large ovocytes in the central part of the gland but with much interstitial tissue and a cortex which appears to be relatively quiescent in that it consists of many ovogonia and only small numbers of ovocytes. The ovaries of treated animals, on the other hand, are distended with many large ovocytes located in the cortex as well as centrally. The interstitial tissue and the residual nests of ovogonia in the cortex are reduced to relative insignificance. The whole histological picture is that of recent enlargement of numerous ovocytes in the cortex of the gland.

The adrenal seems to be somewhat restored by administration of cortical extract in that the cortical cells seem larger, and definitely to contain more lipid material as shown by treatment with osmic acid. The medullary cells in the treated animals constitute a smaller proportion of the gland. Whether this is relative only, due to enlargement of the cortical cells or to actual reduction of the size of the medullary cells has not been determined.

There is no effect on pigmentation, growth, differentiation or upon the thyroid gland. The effect on the male gonad has not been studied.

6005

The Examination of Urine for Indol.

STUART L. VAUGHAN. (Introduced by R. S. Hubbard.)

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Recently it has been suggested¹ that failure of the liver to conjugate indol may lead to an excretion of that substance in the urine. It was therefore decided to investigate the probable value of the demonstration of indol in the urine as a method of determining the relative efficiency of liver function. In addition to a series of patients with clinical evidence of liver disease others showing marked signs of intestinal putrefaction were studied because it was felt that the large amounts of indol formed in such conditions might overtax the detoxifying power of the liver and so lead to the excretion of the unaltered compound in the urine. Simultaneously

¹ Kendall, A. I., *Arch. Path.*, 1931, **12**, 95.

the question of the interference of indol with the usual test for urobilinogen was investigated; for if appreciable amounts of indol are excreted in the urine, positive tests with Ehrlich's reagent would be obtained which would be indistinguishable from the reaction given by urobilinogen.

In this investigation a sensitive qualitative method based upon the reaction between Ehrlich's benzaldehyde reagent and indol and upon the solubility of the latter compound in petroleum ether was used. Urine was treated with barium chloride to remove bilirubin, filtered and the filtrate tested with Ehrlich's reagent according to the technique of Wallace and Diamond.² If a positive reaction was found which might theoretically be due to indol, to urobilinogen, or to various other compounds, 3 cc. of petroleum ether were added to 15 cc. of the filtrate and the tube inverted several times. The ether layer was then removed with a pipette and tested with Ehrlich's reagent for the presence of indol. Preliminary investigations of the degree of sensitiveness of this method showed that if crystalline indol were added to water or to urine a positive reaction was obtained both in the original solution and in the ether extract made as described when the concentration was one part in 800,000 or more. It was also shown that indol could be completely removed by repeated extraction with petroleum ether when the concentration was much higher than this.

The urine of 3 groups of patients was examined by this technique. The first consisted of 100 hospital patients selected at random. All manner of disease entities were represented. The second consisted of 25 persons whose urine contained large quantities of indican, presumably as a result of marked intestinal putrefaction. In the third group, consisting of 22 patients, a disturbance of liver function was thought to be present. Nineteen cases were jaundiced and showed bilirubin in the urine. Diagnoses made clinically, at operation or by post mortem examination, included acute arsenical hepatitis, acute catarrhal jaundice, subacute yellow atrophy, obstructive jaundice and hemolytic jaundice. In 2 cases jaundice was of undetermined origin. In most of the cases the urine was examined repeatedly during the course of the illness. In no specimen of urine examined was a positive reaction obtained in the ether extract. In no instance, therefore, was indol present in a concentration greater than 1 part in 800,000.

In addition to the test for the presence of indol the urine, after it had been extracted with ether, was in every instance tested with

² Wallace, G. B., and Diamond, J. S., *Arch. Int. Med.*, 1925, **35**, 698.

Ehrlich's reagent according to the technique of Wallace and Diamond. Urine samples which gave a positive reaction before extraction gave the same test after petroleum ether had been used. Petroleum ether, therefore, would apparently serve as a satisfactory method for separating indol from urobilinogen if the former compound occurred in urine. The studies show, however, that need for such a separation occurs seldom, if ever.

In this series containing a fairly large number of patients with intestinal stasis and with jaundice due to various causes, indol could not be demonstrated by a very sensitive qualitative test. While a study of a larger number of extreme conditions might yield some positive results, it is hardly possible that the excretion of indol can serve as a satisfactory method of demonstrating impairment of liver function.

6006

Composition of Bone. XIII. Direct Gravimetric Determination
of Ca, Mg, and PO_4 .

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The literature contains few analyses of bone accurate enough to serve for the determination of the molecular constitution of the calcium phosphate present. The deviations from the "theoretical" composition obtained by previous investigators have usually been ascribed either to shortcomings of the analytical methods employed or to experimental error. Shear and Kramer¹ pointed out the possibility that these discrepancies may have been due to actual variations in the composition of bone; this possibility has not as yet been ruled out.

It is therefore desirable to obtain data by methods capable of differentiating between these possibilities. The standard macro analytical procedures are not suitable for this purpose.

An extended study was made of the determination of Ca, Mg, and PO_4 . Each constituent was studied both in pure solutions and in mixtures. It was found that calcium was completely precipitated in solutions just yellow to thymol blue (pH 3), and that it was completely separated from Mg at this pH. Although both PO_4 and Mg

¹ Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1928, **79**, 125.

were present, the Ca precipitate was not contaminated by magnesium phosphate. The Ca was weighed as $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$.

PO_4 and Mg were both weighed as $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ after reprecipitation.

The effect of varying a number of conditions was studied: volume, temperature, pH, amount of precipitate, excess of reagents, the effect of the presence of citrate, proteins, etc.

Several hundred analyses were performed. With the technique finally adopted, results were obtained for each constituent, in pure solution, that were reproducible to 0.3% and that had an accuracy of 0.3%; equally satisfactory results were obtained in mixtures of all 3 constituents. For solutions containing Ca, Mg, and PO_4 in the proportions in which they occur in bone the procedure may be summarized as follows:

Calcium—Add HCl until the solution is red to thymol blue. Add sufficient oxalic acid to give an excess of between 40 and 140%. To the hot solution add dilute NH_4OH until the full yellow color is obtained. Digest for 3 hr. Filter on a weighed Jena crucible. Wash with dilute NH_4OH . Dry at 105°C . for 1 hr. Cool and weigh as $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$.

Magnesium—Evaporate the Ca filtrate to about 75 cc. Add HCl until a clear red solution is obtained. Add enough H_3PO_4 to give from 10 to 50 times the equivalent of the magnesium present. Add NH_4OH slowly to the blue color; then add NH_4OH in excess. Let stand overnight. Decant and redissolve in HCl. Add enough H_3PO_4 to make the phosphate from 5 to 25 times the Mg equivalent. Reprecipitate as before by adding NH_4OH very slowly. Let stand overnight. Filter on a weighed Jena crucible. Wash with cold, dilute NH_4OH . Then wash with alcohol (redistilled from alkali) and finally with ether (redistilled from alkali). Dry at 37°C . for 1 hr., cool and weigh as $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$.

Phosphate—Evaporate the Ca filtrate to about 100 cc. Add HCl to a red color. Add enough MgCl_2 to give from 2 to 4 times the equivalent of the phosphate present. Precipitate by slow addition of NH_4OH as in the Mg determination. Let stand overnight. Decant and redissolve in HCl. Add enough MgCl_2 to make the Mg about twice the phosphate equivalent. Add NH_4OH very slowly to precipitate, as before. Let stand overnight. Filter, wash and weigh as in the Mg determination.

Results obtained with known solutions by means of these procedures are illustrated in the table. When large amounts of Mg were taken for analysis, an accuracy of 0.3% and a reproducibility

of 0.3% were obtained, as in the Ca and PO_4 analyses. When one-tenth this amount of Mg was taken for analysis, as in the case illustrated in the table, the same absolute accuracy in milligrams was obtained; the per cent error was of course 10 times as great for a precipitate of 0.03 gm. as for one of 0.3 gm.

TABLE I.
Analysis of Known Solutions.

pH of Ca pptn. = 3.0.

Constituent	calc.	Pure Solution		Mixture of Ca, Mg, PO_4	
		wt. of ppt.	dev.	wt. of ppt.	dev.
Ca	0.3924	gm.	gm.	gm.	gm.
		0.3915	—0.0003	0.3927	—0.0010
		0.3907	—0.0011	0.3923	—0.0004
		0.3931	+0.0013	0.3946	+0.0009
				0.3939	+0.0002
		0.3918	±0.0009	0.3936	—0.0001
				0.3941	+0.0004
				0.3937	±0.0005
Reproducibility =		0.2%		0.1%	
Accuracy =		0.2%		0.3%	
PO_4	0.3723	0.3721	+0.0009	0.3711	0.0000
		0.3704	—0.0008	0.3716	+0.0005
		0.3710	—0.0002	0.3705	—0.0006
		0.3712	±0.0006	0.3711	±0.0004
		0.2%		0.1%	
Reproducibility =		0.2%		0.1%	
Accuracy =		0.3%		0.3%	
Mg	0.0299	0.0296	0.0000	0.0304	+0.0002
		0.0296	0.0000	0.0300	—0.0002
		0.0295	—0.0001		
				0.0302	±0.0002
		0.0296	±0.0000		
		0.0%		0.7%	
Reproducibility =		0.0%		0.7%	
Accuracy =		1.0%		1.0%	

When proteins were present in the solution, even in small amount, results of this degree of accuracy were not obtainable.

6007

Increased Growth of a Population of Yeast Obtained with Inosite.

OSCAR W. RICHARDS. (Introduced by L. L. Woodruff.)

From the Osborn Zoological Laboratory, Yale University, New Haven.

Following Eastcott's¹ identification of Bios I as inactive inosite it seemed desirable to determine whether or not the medium used in

¹ Eastcott, E. V., *J. Phys. Chem.*, 1928, **32**, 1094.

the investigation of the growth of a population of yeast² might be improved by the addition of this substance. This was done with Williams's medium³ and slightly increased growth (106-116% of that of the control) was obtained with the addition of 0.1-0.4 mg./cc. of inosite, but decreased growth (down to 75% of the control) was found on the addition of 1.0-1.6 mg./cc. of the culture medium.

This increased growth is definite and contrasts with the experiments of Williams, Warner and Roehm⁴ and Narayanan,⁵ who find that the addition of inosite did not increase their yield of yeast. It is difficult to understand this result as Williams *et al* used the same medium and the same culture of yeast as was used in my experiments. The only difference was that my strain of yeast comes from a single cell of yeast No. 2335 (Am. Type Culture Coll.)⁶ and that we are probably not using the same brands of chemicals in the medium. Later Williams and Bradway⁷ obtained a transfer of the yeast used in Miller's laboratory by Miss Eastcott and found that this transfer gave increased growth when 1 mg. of inosite was added to 12 cc. of their medium.

The findings of Williams and myself emphasize the difference between one strain of a species of yeast and a pedigreed strain obtained from an isolated single cell of the same species. The following experiments point out a resolution of this dilemma.

Other experiments have also given increased growth when small amounts of inosite have been added. Adding inosite to the culture medium without asparagine gave the following increases in growth expressed as percentages of the control (no added inosite): for the equilibrium number of cells of the first cycle of population growth 0.1 mg./cc. 192%, 0.5 mg. 103%, and 1.0 mg. 90%; for the equilibrium of the second cycle of growth 0.1 mg./cc. 113%, 0.5 mg. 109%, and 1.0 mg. 104%. Adding inosite to the medium containing Eimer and Amend's asparagine the increases are for the equilibria growth of the first and second cycles respectively 0.1 mg./cc. 131%, and 128%, and for 0.5 mg. 164% and 118%. When the

² Richards, O. W., *J. Gen. Physiol.*, 1928, **11**, 525; 1932, to be published.

³ Williams, R. J., *J. Biol. Chem.*, 1920, **42**, 259; Wilson, J. L., and von der Ahe, F. H., *Ibid.*, 1927, **40**, 227.

⁴ Williams, R. J., Warner, M. S., and Roehm, R. R., *J. Am. Chem. Soc.*, 1929, **51**, 2764.

⁵ Narayanan, B. T., *Biochem. J.*, 1930, **24**, 6.

⁶ Furnished to me through the courtesy of Prof. F. W. Tanner. The isolation was made in 1928 (Richards, O. W., *J. Phys. Chem.*, 1928, **32**, 1865, and 2).

⁷ Williams, R. J., and Bradway, E. M., *J. Am. Chem. Soc.*, 1931, **53**, 783.

medium contains Merck's asparagine the differences in the amount of yeast present at the end of the second growth cycle are for 0.1 mg./cc. 135%, and for 0.5 mg. 115%. These figures come from series 86 and are in addition to the data given above for series 91. The inosite added was obtained from the Eastman Kodak Co., who list it as ash-free. This increase in yield seems to be the result of a stimulant rather than the addition of an essential substance because the rate of growth is greater and the retarding influences appear earlier when this material is present. These differences depend on the kind of asparagine in the medium and indicate that the different brands of asparagine contain a substance which has a much greater effect on the growth of this yeast than does inosite. The isolation and identification of this substance will be published elsewhere.⁸

The recent studies of Williams⁹ and his co-workers suggest again the conclusions of Henneberg¹⁰ and Tanner¹¹ that different yeasts have different nutritional requirements and material serving as a "Bios" for one yeast need not do so for another. These authors indicate that they know of the existence of some 7 "nutrilites" (including inosite) as they choose to call these substances¹² which stimulate the growth of yeast. The rapidly increasing list of these yeast foods indicate that such a *special name* for them is unnecessary.

6008

Studies on Transmissible Lymphadenosis of Mice.*

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A new transmissible strain of malignant lymphadenosis isolated at the Henry Phipps Institute has been observed to possess the following properties.

⁸ In mms. to be published.

⁹ Williams, R. J., and Truesdale, J. H., *J. Am. Chem. Soc.*, 1931, **53**, 4171.

¹⁰ Stated by Tanner, F. W., *Chem. Rev.*, 1925, **1**, 397.

¹¹ Tanner, F. W., Devereux, F. W., and Higgins, E. D., *J. Bact.*, 1926, **11**, 45.

¹² Williams, R. J., *Science*, 1928, **67**, 607.

* This investigation has been supported by a Fund for the Study of Leukemia and Related Diseases.

It can be passed to mice of the stock in which it originated but not to other stocks. About 12% of mice of the same stock, and about half of the closely inbred relatives of the mouse from which this strain was derived were susceptible on intravenous inoculation, but the disease could not be transmitted to 2 unrelated stocks of mice.

The disease caused by intravenous inoculations of emulsions of lymph nodes and spleen resembles the spontaneous disease very closely. Both are characterized by a systemic enlargement of the lymph nodes and splenomegaly, associated with massive lymphoid infiltrations in organs such as bone marrow, liver, kidney, and ovary, assuming in the latter 2 organs tumor-like proportions. In spite of the intravenous route of injection and the massive infiltrations, the number of lymphocytes in the circulating blood was within normal limits or was only slightly increased. The percentage of lymphocytes was likewise within normal limits. Many cells seen in smears, however, were an unmistakable evidence of the disease process. These were medium or large lymphocytes with scant but intensely basophilic cytoplasm and hyperchromatic nuclei. A few cells contained, instead of nuclei, globular or angular material staining red with Romanowsky dyes, this being presumably the disintegrating nucleus. There were also, apparently intermediate between these and immature lymphocytes, lymphocytes with atypical chromatin structure.

Thus the new strain differs from the strains of transmissible lymphoid leucemia of mice previously described¹ in the morphological character of the lymphocytes involved. It differs also in the biological behavior of the immature lymphocytes, in that they invade the organs more extensively, do not invade the blood stream in considerable numbers, and do not affect unrelated mice.

When non-susceptible mice are exposed to X-rays before inoculation, a majority of them will develop lymphadenosis. This observation fully confirms that of Krebs and his associates.²

Quantities of radiating energy much below the lethal dose are sufficient to bring about this change. The dose of X-rays fatal to mice within about 15 days was approximately 600r to 700r; quantities as small as 200r were sufficient to make them susceptible to the strain of transmissible lymphadenosis investigated. The increased susceptibility lasted for at least 42 days; that is, mice could be successfully inoculated from 1 to 42 days after irradiation.

¹ Furth, J., and Strumia, M., *J. Exp. Med.*, 1931, **53**, 715.

² Krebs, C., Rask-Nielsen, H. C., and Wagner, A., *Acta Radiologica*, 1930, Suppl. to Vol. 10.

After 4 successive passages in irradiated mice the disease still failed to effect non-irradiated mice of the same insusceptible stock, but could still be transmitted to non-irradiated members of the susceptible stock.

On subcutaneous inoculation of irradiated mice there was tumor formation at the site of inoculation (lymphosarcoma). This was not accompanied by systemic enlargement of the lymph nodes nor by an involvement of the blood.

These observations together with those reported by Richter and McDowell³ and by Furth and Strumia¹ show that: (a) Lymphadenosis, aleucemic as well as leucemic, resembling closely the spontaneous disease, is best reproduced by intravenous inoculation. (b) Tumor formation (lymphosarcoma) may be produced by the material that causes systemic lymphadenosis when introduced by routes other than the intravenous. (c) Various strains of transmissible lymphadenosis differ from each other in the type of involvement observed (leucemic or aleucemic, intensity of infiltration, etc.). They differ also in their genetical behavior inasmuch as some strains may be transmitted to any stock of mice (Furth and Strumia), and some only to related mice (McDowell and Richter and the transmissible strain described here). The strain of Krebs and his associates can be transmitted only to mice whose resistance is lowered by irradiation. (d) These differences are dependent mainly on the character of the malignant lymphocytes, which retain their original properties after numerous passages.

6009

Specific and Non-Specific Cell Polysaccharides of the Human Type
of Tubercle Bacillus, H₃₇.*

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From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

Six hundred gram portions of ground, defatted human type (H₃₇) tubercle bacilli† were percolated with dilute acetic acid containing

³ Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **52**, 283.

* Carried out under a grant from the National Tuberculosis Association and with the aid of the Harkness Research Fund, Presbyterian Hospital, New York.

† Supplied through the courtesy of the H. K. Mulford Biological Laboratories, Glenolden, Pa.

0.5% of phenol until the neutralized effluent no longer reacted strongly with antiserum. The solutions were concentrated to small bulk *in vacuo* and precipitated with several volumes of alcohol. Further fractionation involved precipitation with glacial acetic acid, with alkali and alcohol, removal of the glycogen with saliva, removal of material precipitable with barium hydroxide (phosphates and a fraction [A]), separation of the residue into 3 main portions, (B_1) soluble in 85% methyl alcohol; (B_2) soluble in 75-85% methyl alcohol, and (C) insoluble in 75% methyl alcohol. Each portion was further purified by fractionation with glacial acetic acid or by special procedures which will be described in a more detailed communication. Fractions B_1 and B_2 are perhaps identical. Properties of the fractions are given in Tables I and II.

It is evident that at least two specific polysaccharides are present,

TABLE I.
Properties of Tubercle Bacillus H_{37} Polysaccharides.

Fraction	$[\alpha]_D$ degrees	Acid Equiv.	N %	P %	Redg. Sugars on hydrol. as glucose %	Basic Ash %
516/17 C	+95	6200	0.1		87	0.2
518/19 C	+85	7250	0.1	0.2		0.3
517 B*	+10	2800	0.7			0.5
518 B_1	+25	2200	0.8		61	
518 B_2	+26	1600		0.9		
519 B_1	+41	2100	0.6	1.0		0.9
519 B_2	+35	2800	0.6	0.2		0.4
518/19 A	+81	1500	0.7	1.8		2.8

* Separation into B_1 and B_2 was not undertaken in this case.

TABLE II.
Serological Reactions of H_{37} Polysaccharide Fractions.

Fraction	Dilution 1 to	5807A Anti- H_{37} Serum*	Serum absorbed with fraction C	517 B	Bovine C†
516/17 C	50,000	+++	—	(+±±)	(±)
518/19 C	50,000	++++±	—	(+±±)	
517 B	50,000	+++++	++	(±)	(+±)
518 B_1	100,000	++++			(+)
518 B_2	50,000	++++±			(+)
519 B_1	50,000	++++±	+±	(±)	(++)
519 B_2	50,000	++++±	++	(+±)	
518/19 A	50,000	++±	+	(+±±)	
Bovine C				(+±±)	(—)

* Supplied through the courtesy of the H. K. Mulford Biological Laboratories, Glenolden, Pa. All fractions recorded in this paper reacted at dilutions of 1:2.5-4 million (highest dilution tested) with this serum. () indicates after centrifugation.

† Bovine type bacillus C fraction.

each reacting with different antibodies. One of these, with the high optical rotation and low degree of acidity, resembles the chief polysaccharide of the bovine and avian type cells (unpublished results) and is perhaps identical throughout the whole group. It yields *d*-arabinose and mannose on hydrolysis. The low-rotating, weak polysaccharide acid of greater solubility is present, if at all, in far smaller amount in the bovine and avian type cells. It readily yields *d*-arabinose in crystalline form on hydrolysis. Mannose does not appear to be present. In addition the bacilli contain at least one other polysaccharide acid. Whether or not this is independently specific, or reacts with antiserum on account of an admixture with the other polysaccharides is uncertain. The results reconcile in a certain measure the divergent findings of Laidlaw and Dudley¹ and Mueller,² the method used by the former workers evidently resulting in a relative concentration of the high rotating specific polysaccharide, while Mueller's process appears to have concentrated material rich in the low rotating specific fraction. Masucci, McAlpine and Glenn's product from tuberculin³ is also a mixture, judging by the properties given.†

6010

Chemotherapy of Experimental Spotted Fever.

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From the Department of Medicine, University Hospital, University of Minnesota.

No specific treatment has yet been developed for diseases caused by rickettsia. Because of the nature of the vascular lesions, and because of the localization of the virus in the endothelium, it would appear that typhus and spotted fever were especially amenable to intravenous chemotherapy. Studies¹ have shown that injections of

¹ Laidlaw and Dudley, *Brit. J. Exp. Path.*, 1925, **6**, 197.

² Mueller, *J. Exp. Med.*, 1926, **46**, 9.

³ Masucci, P., McAlpine, K., and Glenn, J., *Am. Rev. Tuberc.*, 1930, **22**, 669, 678.

† In initiating this investigation the senior author was aided by Dr. Marie Maxim. Since she has assumed responsibility, in an entirely unauthorized communication (*Biochem. Z.*, 1930, **223**, 404), for the results up to the time of her severance from the work. Since the method used had previously been developed in this laboratory and since both methods and findings are now altered, the writers feel further reference to Mrs. Maxim on their part to be unnecessary.

¹ Reimann, H. A., *J. Imm.*, 1930, **18**, 153.

various colloidal substances have a definite effect in prolonging the incubation time and of modifying the subsequent course of experimental typhus fever, if injected shortly after inoculation of the virus. Injection of the same substances after the onset of fever was ineffective. It was concluded that the injection of colloidal substances in the former case probably stimulated the endothelial cells to a defense activity sufficient to delay and modify the course of infection.

Numerous drugs administered intravenously and otherwise have been tested in the treatment of rickettsial disease clinically and experimentally. Salvarsan, chlorine solutions, colloidal silver preparations, novasural, antimony salts, quinine salts, mercurochrome and other substances have been tried. The results were uniformly disappointing. In view of these repeated failures it seemed of value to test some of the newer drugs which have been developed for chemotherapeutic purposes. Germanin (Bayer 205),* metaphen,* triphal (organic gold compound),* and tryparsamide were tested.

The strain of virus used in these experiments was derived from a patient in Minnesota, and was identified as an exceptionally mild type of Rocky Mountain spotted fever by Reimann, Fisher and Ulrich.² After the inoculation of 2 cc. of blood containing the virus intraperitoneally into guinea pigs, fever developed quite regularly on the 6th or 7th day and lasted from 2 to 6 days. None of the animals died from infection. A sample temperature curve in an untreated animal is shown in figure 1 B. In all experiments, the drugs were injected on the first day of fever, intravenously into the heel vein, intraperitoneally or subcutaneously. Attempts were made to give full therapeutic doses calculated on the basis of body

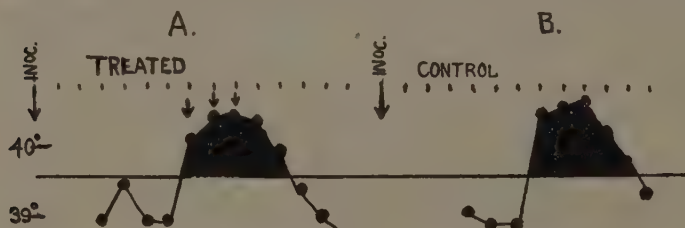


FIG. 1.

Temperature curve of spotted fever in (A) a guinea pig treated on 3 successive days with 0.02 gm. of tryparsamide intravenously, and (B) of an untreated control animal.

* We are indebted to the Winthrop Chemical Company of New York City for supplying these drugs.

² To be published.

weight. Injections were usually repeated on subsequent days and occasionally twice a day. Thirty-one animals were treated and 39 served as controls.

Tryparsamide was given in doses of 0.05 gm. per kilo intravenously to 3 guinea pigs, intraperitoneally in 2, and subcutaneously in 1. A record of a guinea pig treated intravenously is shown in figure 1 A. Triphal was given in doses of 0.125 gm. to 6 animals intravenously. Germanin was given intravenously to 11 guinea pigs in doses of 0.2 gm. and metaphen in 1 cc. doses to 6 animals intravenously and to 2 subcutaneously.

To date we have observed the febrile reaction in 140 guinea pigs.

Results. In none of the animals thus treated could any effect of therapy be detected in the temperature curve. In all cases the temperature curve differed in no material respect from that of the untreated control animals.

Conclusion. Germanin, metaphen, triphal, and tryparsamide had no effect on the course of spotted fever in guinea pigs, when administered therapeutically.

6011

Penetration of Antipneumococcic Horse Serum into the Consolidated Lung in Lobar Pneumonia.

T. HUNNIOUTT AND W. SUTLIFF. (Introduced by Robert N. Nye.)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, Boston, Massachusetts, and from the Department of Medicine, Harvard Medical School.

In analyzing the action of antipneumococcic serum in the treatment of lobar pneumonia, the question of its penetration of the pneumonic lung is often raised. The clinical observation that specific therapy is effective early in the disease and ineffective late in the disease has sometimes been explained on the basis of the accessibility of the pneumonic area to circulating antibodies in the initial stages of consolidation and a lack of penetration after complete consolidation has taken place. Studies of the circulation in the pneumonic lung have usually supported this view. Mallory¹ believed from the study of pathological histology, that in the stage of gray hepatization the air sacs became distended and the capillaries compressed so that they no longer appear engorged. Kline and

Winternitz,² using vital staining methods with trypan blue, concluded that there is a marked impairment of circulation in the pneumonic lung, resulting from the wide distribution of capillary fibrin thrombi formed early in the consolidated area, and that this impairment of circulation in the pneumonic lung seriously interferes with the action of intravenous therapy upon the local lesion. Gross,³ using injections of a barium sulphate emulsion in gelatine, noted that the consolidated lung of lobar pneumonia shows poor injection with compensatory injection in the non-consolidated lung. Coryllos and Birnbaum,⁴ however, using iodized oil for arterial circulation and Ringers' solution and india ink for capillary circulation in the consolidated lung of the dog, concluded that "Only the capillary circulation was involved. Circulation in the pulmonary arterial tree is not affected. The capillary impairment is not complete."

It was felt that inability to inject the vascular bed in human pneumonic lungs was not conclusive evidence of marked impairment and the possibility remained that intravenously injected serum might penetrate into the consolidated area. The apparent maintenance of nutrition in the lung tissue, as evidenced by its quick return to normal after the crisis without abscess formation, suggests that no complete circulatory embarrassment occurs.

A number of workers have detected intravenously injected horse serum in the body fluids and organ extracts by means of specific precipitating antihorse sera. Hamburger and Moro,⁵ Uhlenhuth and Weidanz,⁶ Dean, Goldsworthy, and Ten Broeck,⁷ among others, observed its presence in the blood serum. Menkin⁸ studied the fixation of horse serum at the site of inflammation in the skin and peritoneum of the rabbit. He found the horse serum in greater concentration at the site of inflammation than in normal tissues.

In the present study the blood serum, and extracts of the consolidated and unaffected lung and of the liver, kidney, and spleen, all obtained at post mortem from patients with lobar pneumonia who

¹ Mallory, F. B., *Principles of Pathological Histology*. Philadelphia, W. B. Saunders Co., 1914, 472.

² Kline, B. S., and Winternitz, M. D., *J. Exp. Med.*, 1915, **21**, 311.

³ Gross, L., *Canadian Med. Assn. J.*, 1919, **9**, 632.

⁴ Coryllos, P. N., and Birnbaum, G. L., *Arch. Surg.*, 1929, **19**, 1346.

⁵ Hamburger, F., and Moro, E., *Wien. Klin. Woch.*, 1903, **16**, 445.

⁶ Uhlenhuth, P., and Weidanz, O., *Kraus and Levadittis Handbuch*, Jena, Fischer, 1909, **2**, 819.

⁷ Dean, H. R., Goldsworthy, N. E., and Ten Broeck, C., *J. Immun.*, 1930, **18**, 95.

⁸ Menkin, V., *J. Exp. Med.*, 1930, **52**, 201.

had received intravenous antipneumococcic serum therapy just previous to death, were examined for their content of horse serum. The 3 patients died 11, 4½, and 9½ days respectively following the onset of pneumonia.

Methods. 1. *Preparation of organ extracts.* Organs were removed at autopsy, 3 hours, 1½ hours, and 28 hours, respectively, after death. Contamination with tissue fluid and with pleural or peritoneal fluids was avoided, as far as possible. The organs were placed on clean dry towels and the surfaces wiped free of exuded blood and tissue fluid. They were then sectioned and blocks of centrally located tissue were selected. In the case of the lung, specimens of gray hepatization, red hepatization, and normal lung were taken. Blood from the heart was secured, in order to obtain serum. Forty grams of each of the selected tissues were ground with sand in a mortar, first dry and then with the addition of sterile normal salt solution until 20 cc. had been added. This mixture was centrifuged and the clear supernatant fluid was filtered through a Berkefeld V candle, under sterile conditions. These extracts were kept in cotton-stoppered test tubes at ice box temperature.

An antihorse rabbit serum was used to detect the presence of horse serum in the organ extracts. Rabbits were immunized with 6 doses of 0.5 cc. normal horse serum, subcutaneously, at weekly intervals, and bled 8 days after the last dose. The sera from several animals were pooled, passed through a Berkefeld filter and kept in sealed tubes. This serum gave a precipitate with normal horse serum in a dilution of 1:15,000 and with Felton's antipneumococcic serum in a dilution of 1:10,000. Two-tenths cc. of varying dilutions of the extracts were added to equal amounts of antihorse rabbit serum, incubated for 2 hours at 37°C., and read after over-night ice box storage. Pooled normal rabbit serum, obtained from the same rabbits before immunization, and normal salt solution were used as controls. Soluble specific substance was detected in the organ extracts in a similar manner, using Types I, II, and III antipneumococcic sera in each instance.

The titres of demonstrable horse serum in the post mortem sera and organ extracts are shown in the accompanying table. Liver extracts were unsatisfactory because of non-specific reactions in the normal saline controls. Tests with extracts of spleen were omitted from the table, as they gave nonspecific reactions with both normal serum and saline controls. A comparison between unaffected and consolidated portions of the same lung shows that in 2 cases the same amount of horse serum was present; in the third (Case III),

Case No. Age	Time of Treatment		Amount Anti- pneumococcus serum cc.	Autopsy		Organ	Culture	Tests for Horse Serum with Anti-Tests for S.S.S. with			
	First dose	Last dose		Date Time	Hours after Death			Antihorse Rabbit Serum	Horse Rabbit Serum Normal Rabbit Serum	Saline Control	Specific Typing Sera Type I
	At Autopsy										
I-33	3:30 P.M.	4/8 1:00 A.M.	276	4/13 1:00 P.M.	3	Post Mortem Serum	Pn. Type I	1:128	0	0	—
						Normal	Negative				
						Lung		1:32	0	0	—
						Gray					
						Hepati- zation L.L.L.	Pn. Type I	1:32	0	0	1:128†
II-66	10:00 A.M.	3/7 1:00 P.M.	30	3/7 4:00 P.M.	1½	Kidney	—	1:32 1:4	0 0	0 1:16*	0† 1:8†
						Liver	—				
						Post Mortem Serum	Negative	1:128	0	0	0†
						Normal					
						Lung	Pn. Type I	1:16	0	0	1:8†
III-45	12:00 P.M.	2/16 2:00 A.M.	295	2/24 4:00 P.M.	28	Gray		1:16 1:4	0 ±	0 1:16*	1:8† 1:8†
						L.L.L.	—				
						Kidney	—				
						Liver	—				
						Post Mortem Serum	Pn. Type I	1:256	0	0	0†
III-45	12:00 P.M.	2/23 2:00 A.M.	295	2/24 4:00 P.M.	28	Normal	Negative	1:64	0	0	0†
						Lung					
						Gray					
						Hepati- zation R.L.L.	Pn. Type I	1:32	0	0	1:8†
						Kidney	—	1:128 1:16*	0 1:16*	0 1:16*	— 0†
* Nonspecific Reactions. † No reactions were obtained with Type II and Type III.											

* Nonspecific Reactions. † No reactions were obtained with Type II and Type III.

however, there was a slightly higher titer of precipitinogen in the non-consolidated portion. The concentration of horse serum in the kidney was the same as that in the unaffected lung in each case.

Type specific pneumococcic antigen was demonstrated in considerable concentration in all the consolidated lung extracts. It was also found in lower titer, once in normal lung extract, once in kidney extract, and twice in liver extract.

Discussion and summary. It has been shown that horse serum administered intravenously before death to patients with lobar pneumonia is present after death in the consolidated lung in a concentration similar to that in which it is found in unaffected organs. The short time elapsing between death and autopsy in 2 of these cases makes it seem likely that the post-mortem finding represents a condition present during life. No evidence was obtained as to the mode of penetration of the horse serum. Due to the lack of conclusive proof of circulatory occlusion in the pneumonic lung it is possible that the foreign serum entered and was contained wholly within the blood vessels. On the other hand, the foreign serum may have diffused through cellular membranes to such an extent as to penetrate a region in which little or no blood circulation was active. No increase in concentration was found in the consolidated lung which would correspond to the increase noted by Menkin⁸ in local inflammatory areas, but this might be explained by the local impairment of circulation. In any case, the finding of the foreign serum within the consolidated lung, together with the penetration of foreign serum into pleural exudates, as shown by Finland,⁹ suggests that therapeutic sera are active in lobar pneumonia at the site of the lesion.

Such penetration of therapeutic serum into the consolidated lung is of theoretical and practical significance. In the experiments above, the consolidated lung and other organs containing horse serum also contained type-specific precipitinogen, indicating that the latter was not completely neutralized. The relative lack of therapeutic effect of antipneumococcic sera late in the course of the disease may be due, not to the lack of penetration of antibodies, but to this accumulation of type-specific antigen. It seems possible that antibodies, if present in sufficient concentration, would neutralize all of the type-specific antigen. Whether or not the use of high concentrations of antibodies with a possible complete neutralization of the type-specific antigen would be effective in all advanced cases can not be judged without actual trial since other factors, such as antigens or

⁹ Finland, Maxwell, *J. Exp. Med.*, In Press.

toxins not neutralized by the antiserum or irreparable toxic damage, may be present. A logical justification, however, for attempts to produce very potent antibodies is apparent.

6012

A Quantitative Relation Between the Chloride and Acid Concentrations in Gastric Juice.

FRANKLIN HOLLANDER.

From the Department of Physiology, New York Homeopathic Medical College.

Examination of the literature on the chloride content of gastric juice shows that the usual method for the study of such data depends on their being plotted against the time which elapsed between stimulation and collection of the fluid. Correlation of total chloride and acidity is made by direct visual comparison of such time curves. Regarding the existence of a relation between these 2 variables, 3 different conclusions have been reached: (1) that the total chloride concentration is constant throughout any one experiment. (2) Significant variations do occur, but they are always smaller than the concomitant changes in acidity and bear no relation to the latter. (3) A parallelism exists between such acidity and chloride curves. In the present investigation, decisive evidence is offered for the existence of an exact quantitative relation between the concentrations of acid and total chloride, and an explanation is offered to account for the differing conclusions of other investigators.

Experiments with 5 Pavlov pouch dogs were performed in the usual way. In almost every instance, the chloride values fluctuated within such narrow limits that they gave a distinct impression of constancy. In only 2 of the 19 experiments was the total range greater than 10mM. When the 2 variables were each plotted against the time, an unmistakable parallelism of the 2 curves was noted in 12 of the experiments. In the remainder, the occurrence of such a parallel relation may have been hidden by the combined influences of high experimental errors and a small range in acidity values. These experiments, therefore, support the notion of a parallelism between the chloride and acid curves; they also suggest an explanation for the failure of other investigators to observe this relation.

Such a parallelism may be indicative of a quantitative relation

between the chloride and acid concentrations; consequently, these variables were plotted against each other, thus excluding the time factor entirely. In all cases, the points so plotted conformed to straight lines, of which the slopes and the intercepts with the acidity axis were fairly uniform for the 12 experiments. Due to the accidental errors, however, the random variations of the individual points from these lines were rather large. To reduce the influence of these errors, the combined data of all 19 experiments were analyzed statistically. By this method, the observations were in even better agreement with a straight line, a result which is all the more significant because it includes the 7 experiments showing no definite parallelism of the time curves.

To show that the method of collection of the gastric juice had no influence on the result, data obtained from 3 dogs by the *discontinuous collection* procedure (described elsewhere¹) were similarly analyzed. Also, 2 experiments by a group of investigators² who have claimed that the concentration of total chloride is constant, were plotted with exclusion of the time factor. In all cases, the results completely verified the existence of such an exact relation.

Since the graph of total chloride against total acidity is a straight line, a similar relation must exist between the neutral chloride concentration (calculated as the difference between total chloride and acidity) and the acidity. A mathematical analysis of the graphs obtained from both total and neutral chloride values demonstrates that the Rosemann theory of hydrochloric acid formation is incompatible with the observations, whereas the Heidenhain theory, as modified and extended by Hollander and Cowgill,¹ is in excellent accord with them. In fact, the equations describing the experimental graphs have the same form as those derived on the basis of the latter theory. Furthermore, values for the isosmotic concentration (with respect to blood) of hydrochloric acid, calculated from the experimental equation in accordance with the theory, were in good agreement with those found in the literature.

¹ Hollander, F., and Cowgill, G. B., *J. Biol. Chem.*, 1931, **91**, 151.

² MacLean, H., Griffiths, W. J., and Williams, B. W., *J. Physiol.*, 1928, **65**, 77.

Missouri Section.

Washington University School of Medicine, December 9, 1931.

6013

Specificity in Action of Anterior Pituitary of Different Mammals, and Urine of Pregnant Women on Ovary and Thyroid.*

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

During the past 2 years we have compared the action of the anterior pituitary of various species of mammals as well as of the urine of pregnant women on ovary and thyroid gland of the immature guinea pig and have observed typical differences in their effects. These we shall very briefly summarize.

1. Various preparations of *cattle anterior pituitary* (anterior pituitary inoculated in substance, alkali and acid extracts, residue after preceding extraction), all have in common the production of a hypotypical condition of the ovarian follicles, largely, but perhaps not entirely, due to the increased basal metabolism caused by the hyperactivity of the thyroid which these substances induce. They also tend to produce pseudolutein bodies and interstitial gland. Ovulation does not occur except when, after a period of long-continued injections, the effect of the extracts on the thyroid gland has ceased.

2. *Inoculation of anterior pituitary of mature male guinea pigs* accelerates markedly maturation of follicles and may produce a relatively slight amount of interstitial gland and pseudolutein tissue. The administration of this anterior pituitary substance has in many cases been followed by premature ovulation. Acceleration of maturation of follicles and ovulation are therefore the most characteristic effects observed. Variation in the amount of the inoculated material may lead to variations in the intensity of the effects without causing thereby the relation between acceleration of maturation and production of interstitial gland and pseudolutein tissue to be greatly altered.

* These investigations, presented at the May, 1931, meeting of the Missouri Section of this Society, were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

3. *Inoculation of anterior pituitary of young male adult rabbits* accelerates markedly maturation of follicles, but at the same time, it induces formation of pseudolutein tissue and of interstitial gland to a much higher degree than does anterior pituitary of guinea pig. It does not as a rule induce ovulation. At later periods of inoculation we may find the ovary largely filled with interstitial gland.

4. *Inoculation of anterior pituitary of cats* exerts on the whole the same action as rabbit anterior pituitary, but it is apparently somewhat less effective than the latter; therefore somewhat larger quantities need to be administered.

5. *Intraperitoneal injection of urine of pregnant women* converts follicles into pseudocorpora lutea of various sizes and induces production of interstitial gland. During the third week of injection the greater part of the ovary becomes gradually converted into areas of interstitial gland which develop around atretic follicles. Mature follicles are as a rule not produced and ovulation does not take place.

Each of the preparations used exerts specific effects, largely, but not entirely, independent of the quantity of the material used. In general, ovulation is induced in the guinea pig only by anterior pituitary of the guinea pig. As to their effects we can arrange these preparations in the following order: (1) Urine of pregnant women; (2) cattle anterior pituitary; (3) rabbit anterior pituitary; (4) cat anterior pituitary; (5) guinea pig anterior pituitary. (1) and (2) cause the production of the greatest amount of interstitial gland and pseudolutein tissue. (5) mainly accelerates maturation of follicles and may cause ovulation; it produces only little interstitial gland and pseudolutein tissue. (3) and (4) are intermediate in their action.

If we compare with these effects on the ovary, the action of these substances on the thyroid gland, we find that (2) and especially the extracts cause the greatest hypertrophy. However, we must consider the fact that in preparing these extracts a relatively much greater amount of substance of cattle anterior pituitary is used than is possible to inoculate daily into the guinea pig if we use the gland substance as such. (5) has only a very slight effect or none, while the effects of (3) and (4) are again intermediate. As we previously observed injection of urine does not cause any definite hypertrophy of the thyroid gland. To a certain extent the hypertrophying effect of these substances on the thyroid follows therefore a parallel course to their ability to produce interstitial gland and pseudolutein tissue in the ovary; it seems independent of their ability to accelerate maturation of follicles and to produce ovulation. As

to the exceptional position of urine of pregnant women which causes a marked production of interstitial gland and pseudolutein tissue, without causing hypertrophy of the thyroid gland, there are some indications that the active substance in urine may be different from the active principle in the anterior pituitary.

6014

Administration of Theelol to the Immature Brown Leghorn Female.*

J. B. MITCHELL, JR. (Introduced by John Auer.)

From the Departments of Pharmacology and Physiology, Saint Louis University School of Medicine.

The properties of theelol as an estrogenic substance allied to theelin, and the report of atretic degeneration in the ovary of the rat upon continuous administration of theelin¹ suggested the possibility of some action of theelol upon the ovary of the immature fowl.

Using 20 Brown Leghorn pullets, 5 months of age, 5 served as controls (saline injected), and 15 received daily an aqueous solution of theelol intramuscularly. These were in 3 equal lots receiving 2.5, 10, and 20 rat units respectively per day. After 40 days the dosage was double in each lot, and again after 16 days the dose was further increased to 20, 40, and 100 units per bird per day respectively. The birds were inspected and weighed weekly.

As indicated by comb-growth and egg-production, after a total of 96 days of injection, no signs of ovarian stimulation or inhibition were noted. The first bird to lay was a control, followed by 2 birds of the smallest theelol dose, and later by members of the other groups. Laying commenced a week after the heaviest dosage was started, and after 3 weeks, 2 controls, three 20-unit, two 40-unit, and one 100-unit bird was laying. Autopsies revealed only ovaries and oviducts normal for birds of laying age. Thus there was found no inhibition nor stimulation of ovulation by theelol as employed. The problem is being further studied, especially with reference to elimination rate as a possible explanation.

* Expenses were borne in part by a grant from the Committee for Research in Problems of Sex, of the National Research Council. I am indebted to Dr. Doisy and his associates for the theelol used in this experiment.

¹ Doisy, E. A., Thayer, S. A., Levin, L., and Curtis, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 88; Doisy, E. A., Curtis, J. M., and Collier, W. D., *Ibid.*, **28**, 885.

6015

Injection of Avian Anterior Pituitary Substance into the Leghorn Fowl.*

J. B. MITCHELL, JR. (Introduced by John Auer.)

From the Department of Pharmacology, Saint Louis University School of Medicine.

Several workers¹ have attempted to modify the genital system of the fowl by pituitary administration. With the exception of Clark, these have been unsuccessful, either by the oral or by the intraperitoneal route. Walker found the injection of a bovine preparation resulted in inhibition of ovulation in the hen.

Wishing to test the possible effectiveness of avian pituitary substance, the writer employed a technique which consisted in the collection of freshly killed chick heads, immediate dissection of the anterior lobes, and the daily intramuscular injection of these into White Leghorn pullets. In the first experiment, 20 three-months-old birds, divided into 4 equal lots, received for 28 days doses of 1, 2, or 3 anterior lobes. One lot as controls, consisted of saline injected, brain tissue injected, and untreated birds. Weights were periodically taken, and autopsies done. Careful examination of the ovaries and oviducts of these birds failed to reveal any deviations from the controls, although the weights of the injected birds were somewhat less than those of controls.

To extend the range of dosage and time, in a second experiment, using similar material and technique, 2 birds received 10 lobes each, three 5 lobes each, and four 3 lobes each for 56 days. There were 10 controls. Autopsies again revealed no departure of either ovaries or oviducts from controls, the weights again being slightly less than controls.

In a third experiment, 6 hens with a previous trapnest record were injected for 23 days as follows: one received 6 lobes daily, one 4 lobes, and one 2 lobes; 3 were controls, untreated, saline, and brain tissue injected. Excluding eggs probably already in the oviducts, controls produced 18 eggs, and injected birds 10 during the injection period. Variation in the individual records, and the lim-

* From the Department of Zoology, University of Georgia, with the cooperation of the Poultry Department, Georgia State College of Agriculture. The expenses were borne in part by a grant from the Committee for Research in Problems of Sex, National Research Council.

¹ Clark, N. C., *J. Biol. Chem.*, 1915, **22**; Pearl, R., and Surface, F. M., *Ibid.*, 1915, **21**; Pearl, R., *Ibid.*, 1916, **24**; Simpson, S., *Quar. J. Exp. Physiol.*, 1923, **18**; Walker, A. T., *Am. J. Physiol.*, 1925, **74**.

ited material make definite conclusion of an inhibition of ovulation unwarranted.

Without considering further the limits of technique, it appears that with these dosages and methods, there is no evidence of a response of the female genital system of the fowl to avian pituitary substance. It is recognized that such factors as absorption and elimination perhaps play a rôle in the explanation of these results.

6016

Influence of Bladder Extracts and Viosterol on Healing of Fractures and Bone Defects.

GLOVER H. COPHER, J. ALBERT KEY AND EDWARD S. WEST.

From the Departments of Surgery and Biological Chemistry, Washington University School of Medicine, and the Shriners' Hospital for Crippled Children, St. Louis.

Huggins¹ has shown that if a piece of the mucous membrane of the urinary bladder of a dog is transplanted into the abdominal wall of the same animal the transplant forms a cyst lined by bladder epithelium and true bone tends to form in and about the wall of the cyst. We² have confirmed Huggins' observation and have also shown that an autogenous transplant of bladder epithelium may stimulate the repair of defects in normal bone. This was demonstrated by a series of experiments in which comparatively large defects were created in both ulnas of dogs and at the original operation the defect on one side was bridged by a strip of bladder mucous membrane while the similar defect in the other ulna was left as a control. The defect bridged by the bladder transplant tended to heal by deposition of bone while non-union occurred on the control side. Similar results were obtained when scrapings from the mucous membrane of the dog's bladder were placed in fresh ulnar defects in the same animal.

The results of these experiments were so striking that we have performed other experiments in a search for an explanation of the phenomena. It seemed possible to us that the effect of the mucous membrane might be due to a hormone. Accordingly, extracts were made from bladders of dogs and swine and were injected at the site of fractures and bone defects.

¹ Huggins, C. G., *Arch. Surgery*, 1931, **22**, 377.

² Copher, Glover H., and Key, J. Albert, unpublished article.

Fresh mucous membrane, obtained from dog's bladder by stripping it from the muscularis, was titrated by grinding it in a mortar containing sand and normal saline. After standing for awhile to let the sand settle to the bottom of the container, the supernatant fluid containing the macerated cells of the mucous membrane was decanted off and was preserved in an icebox. Two cubic centimeters of this saline extract were injected into the site of experimental fractures of the right ulnae of 5 dogs. The fractures had been made by subcutaneous osteotomy 2 days previously. The fractures made similarly in the left ulna were kept as controls. One week after the first injection another 2 cc. of the extract were injected into the site of each fracture on the right side. The 5 dogs were sacrificed at intervals of 2 to 7 weeks after the first injection had been made. In no instance was union of the fracture influenced by injection of the extract as determined by gross, and microscopic appearance and by X-ray films. Likewise, an alcoholic extract of dogs' bladders was ineffective in hastening union.

In the next series of experiments, defects approximately 1 cm. in length were made in the ulnae of dogs. Fresh pig bladders with fat and connective tissue removed were finely ground and extracted with 70% alcohol (2 cc. per gm.) for 24 hours at room temperature. The mixture was strained through a thoroughly washed chamois and divided into portions representing 12 gm. of bladder tissue each. Alcohol was removed under vacuum over sulphuric acid at room temperature and the residues kept in the desiccator. The residues were taken up in 9 cc. of normal saline solution and 1.5 cc. of the solution was injected into the site of the right ulnar defect in each of 6 dogs. The left ulnar defects were kept as controls. The injections were made at 3 and 4 day intervals until the animals were sacrificed at intervals of from 4 to 8 weeks.

Aqueous chloroform extracts were prepared by treating pig's bladder hash (150 gm.) with 300 cc. of water and 25 cc. of chloroform for 24 hours at room temperature and giving the material an occasional vigorous shaking. The mixture was filtered as in the alcoholic preparation and the extract was preserved in a cold room. Shortly before use, extract representing 12 gm. of bladder was evaporated under vacuum over sulphuric acid at room temperature. The residue was taken up in 6 cc. of normal saline solution and 1½ cc. of this was injected into short ulnar defects as were the alcoholic extracts. The 4 dogs were sacrificed at the end of 8 weeks. Gross, microscopic and X-ray examination of the specimens revealed no difference in the healing of the fractures on the 2 sides.

In another series of dogs 1 cc. of irradiated ergosterol (Viosterol) was injected daily into the tissues around experimental fractures in the same manner as were the bladder extracts. The results of these experiments were also negative. That is, there was no definite difference in the healing on the control and injected sides.

Conclusions. Neither extracts of bladder prepared as described above nor irradiated ergosterol influenced osteogenesis when injected at the site of experimental fractures and bone defects. It is possible that the stimulation of osteogenesis noted when bladder epithelium is transplanted into bone defects is due to a local excess of calcium which is produced by the living bladder epithelial cells. It is also possible that phosphatase is a factor in the production of bone around the bladder transplants.

6017

Exophthalmos Produced by Injections of Acid Extract of Anterior Pituitary Gland of Cattle.

LEO LOEB AND HILDA FRIEDMAN.

From the Department of Pathology, Washington University School of Medicine.

In former investigations¹ we observed that by injections of acid and alkali extracts of anterior pituitary of cattle, it was possible to produce in the guinea pig changes in the thyroid gland identical with those in very pronounced cases of Graves' disease in man, namely, a marked hypertrophy and hyperplasia of the acinus cells, softening and absorption of colloid and irregular shape of the acini, associated often with an almost complete disappearance of the lumen of the acinus. There was also a loss of weight induced by these changes which indicated an hyperactivity of the gland. Silberberg found that administration of KI to a certain extent inhibited these changes. This corresponds to the action of this substance in Graves' disease. Siebert noted a marked rise of the basal metabolism in guinea pigs under the influence of acid or alkali extracts and more recently found that simultaneous administration of KI tends to counteract the effect on basal metabolism. Closs, Loeb and MacKay¹ found the changes in the distribution of organic and inorganic

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

¹ Closs, Carl, Loeb, Leo, and MacKay, Eaton, *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 170.

iodine in the thyroid gland and blood of guinea pigs injected with acid extract similar to those which occur in Graves' disease.

During the winter, 1930-31, we observed that guinea pigs injected with acid extract frequently showed a greater bulging of the eyes than normal guinea pigs. In view of the correspondence of this change to the exophthalmos which is so characteristic of Graves' disease, we followed the alterations in the eyes on a large number of guinea pigs. Our inability to measure these changes accurately or representing them objectively, caused us to hesitate to publish these observations. The method we have adopted recently makes it possible to demonstrate the bulging of the eye in a relatively objective manner.

We selected in each case 2 guinea pigs with similar eye color, similar prominence of eyes, and of similar weight, varying in different pairs approximately between 180 and 210 gm. The similarity of eye color is a necessary precaution, because there exists certain variations in the character of the eyes of individual guinea pigs. Then one of the 2 animals of each pair received daily intraperitoneal injections of $2\frac{1}{2}$ to 3 cc. acid extract of anterior pituitary. After a series of from 4 to 6 injections, the eyes of the injected animals were distinctly more prominent than those of their partners. Thus it was possible by merely comparing the eyes of the 2 partners, to determine correctly in about 70% to 100% of the pairs which one of the 2 animals had been injected. As a matter of routine, a number of observers were shown these pairs and they made a diagnosis independently of each other. As a rule their judgment coincided. In a certain percentage of animals, no distinct difference developed between the 2 partners, or it developed somewhat later. When such a bulging of the eyes had been established it could remain noticeable for a long period of time, if the injections of extract were continued. However, it seems that if the guinea pigs lose a great deal of weight and become weak, this symptom may disappear; it also disappears in complete narcosis and after death. The exophthalmos depends therefore on a functional condition and not on an organic change. Whether it is due directly to hyperthyroidism needs further investigation.

In their first observations, Loeb and Bassett noted that the changes produced by injections of extracts in the thyroid gland of rats are missing or much less pronounced than in the thyroid of guinea pigs. Correspondingly, we found that a bulging eye cannot be definitely obtained in rats.

We may thus consider that in addition to the other similarities between the symptoms of Graves' disease in men and those pro-

duced by administration of preparations of anterior pituitary in guinea pigs we now have established the occurrence of exophthalmos.

6018

Effect of Amytal upon Basal Metabolism in Guinea Pigs.*

WALTER J. SIEBERT AND ERIC W. THURSTON. (Introduced by Leo Loeb.)
*From the Department of Pathology, Washington University School of Medicine,
St. Louis.*

Deuel, Chambers and Milhorat¹ have shown that there was only a very slight lowering of basal metabolism in dogs which had been injected with sodium amytal. A series of experiments was carried out to determine what effect doses of amytal sufficient to cause the animals to remain quiet would have upon the basal metabolism of normal guinea pigs, and upon guinea pigs which had been given various substances which would cause a rise in the basal metabolic rate. We used the isoamylethylbarbituric acid (Amytal—Lilly) which was prepared according to the directions given on the label of the bottle. 0.10 cc. of a saturated solution of the potassium salt of the amytal was injected subcutaneously into guinea pigs which had an average weight of about 400 gm., 5 or 10 minutes before placing the animals on the metabolism apparatus. The guinea pigs remained perfectly quiet throughout the 2-hour period in which they were on the apparatus. No shivering occurred. No ill effects from the use of the amytal and no increased tolerance for the drug were noted. The average basal metabolism of 76 normal guinea pigs without amytal was 3.59 calories per kilo per hour, while in 72 other guinea pigs which had been injected with amytal, the average basal metabolism was 3.52 calories per kilo per hour. Thus the average metabolism of this latter series was approximately 2% below the average of the series which had not received amytal. The maximum and minimum variations are approximately the same in both sets, being plus or minus 10%. Twenty-four guinea pigs were used to determine what effect amytal has upon basal

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

¹ Deuel, Harry J., Jr., Chambers, William H., and Milhorat, Adolph T., *J. Biol. Chem.*, 1926, **69**, 249.

metabolism if various substances are given which cause a rise in the basal metabolic rate. For this purpose 8 guinea pigs were given daily 1 cc. intraperitoneal injections of acid anterior pituitary extract; 4 of these received amytal previous to the period during which the determinations were being made, and in 4 others the determinations were made at the same time without the use of amytal. The metabolism was tested at biweekly intervals for periods of 2 to 3 weeks and the maximum and minimum variations and the average curves of the 2 sets of animals were compared. The maximum rise as well as the average curve and the minimum rise was found to be very slightly lower in the group of animals which had received amytal, but the curve of metabolism was very similar in both types of cases. The variations between the maximum and the minimum rise in metabolism was also smaller in the group which had received amytal.

Eight other guinea pigs were given Armour anterior pituitary tablets (5 grain) daily. Four of these were given amytal, and in 4 others determinations were made at the same time without amytal. The results were compared after biweekly determinations were made in both sets of animals over a 3-week period. In this series the metabolism curve is the same in both sets of guinea pigs. The variations are considerably less in the group which had received amytal, but in this set the maximum and minimum determination, as well as the average curve was very slightly higher in the group which had received amytal. Thyroid substance (Armour, 0.05 grains daily by mouth) was given to 8 guinea pigs, 4 of which received amytal and 4 in which the determinations were made without amytal, the metabolic rate was tested in both sets at the same time at biweekly intervals over a 3-week period, and the results compared. The maximum and minimum values were smaller in the group which had received amytal, the average curve was very slightly less but the type of curve was similar in both sets.

Basal Metabolism after KI with Acid Extract of Anterior Pituitary Glands, Pituitary Tablets and with Thyroid.*

WALTER J. SIEBERT AND ERIC W. THURSTON. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis.

Studies were made upon the effects of combinations of KI with acid extracts from anterior pituitary glands of cattle, with (Armour) anterior pituitary tablets and with thyroid substance. A large number of determinations were made upon 48 guinea pigs at biweekly intervals, using the Haldane open circuit respiratory colorimeter. Subcutaneous injections of amytal were given the animals in order to eliminate movement while the determinations were being made.

In the first set of experiments, 9 animals were given daily intraperitoneal injections of 1 cc. of fresh acid extract of cattle anterior pituitary gland. Nine others were given similar injections and in addition were given 0.05 gm. KI pills by mouth daily. The acid extract injections produced a marked rise in basal metabolism in all the 9 guinea pigs. The basal rate usually reached a maximum of approximately 60% above the normal average within the first 12 days, after which there was usually a gradual decline. The animals which were given the acid extract from fresh cattle glands in combination with KI failed to show any marked rise in the metabolic rate, the average being slightly above that of normal guinea pigs.

In the second set of experiments 9 guinea pigs were given Armour anterior pituitary 5 grain tablets daily over a period of 24 days. There was a fairly constant rise in metabolic rate in all 9 guinea pigs. This reaches a level ranging between 30 and 50% above the normal within 8 to 12 days. After this initial rise there is a fall ranging to 10 to 30% above the normal, after which there is again a gradual and steady rise so that at the end of the 24-day period, the average rate is approximately 70% above the normal. Nine guinea pigs which were given the same dosage of Armour anterior pituitary tablets as above and were given in addition 0.05 gm. KI pills by mouth daily. In this series the average metabolism curve is very similar to the one obtained when the Armour

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

tablets were given alone. There were variations both above and below the average curve obtained in the series which had received the Armour tablets alone, but the majority of the determinations as well as the average curve are somewhat higher in the series in which the KI was administered in combination with the Armour anterior pituitary tablets.

Twelve guinea pigs were used in a series of experiments in which thyroid substance was administered alone in doses of 0.05 grain (Armour thyroid substance) and in combination with KI 0.05 gm. Both drugs were administered daily by mouth. The average curve of the metabolic rates in the set of 6 guinea pigs in which the thyroid substance was given alone is very similar to that obtained when Armour anterior pituitary tablets were administered. There is a fairly sudden rise within the first 8 to 12 days which ranges from 35% to 50% above the normal. This rise is followed by a fall which occurs in all the animals and ranges from 30% to 100%. After this there is a gradual and steady rise so that at the end of the 28 day period in which the animals were observed, the basal metabolic rate reaches a level approximately 50% above the normal rate. The animals which had received KI in combination with the thyroid substance showed in general a similar average curve of the metabolic rate to that obtained in those which had received the thyroid substance alone. There were variations in the determinations in the former series both above and below those in the latter series but the majority of the determinations as well as the average curve in the guinea pigs which had received thyroid substance with KI were slightly higher, especially in the latter periods, than in those which had received the thyroid substance alone.

6020

Does Castration in Guinea Fowls Produce Sexual Differentiation in Plumage and Changes in Head Furnishings?

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Brno, Czechoslovakia.*

In guinea fowl there are no sex differences in shape, pigmentation and pattern of plumage, and but slight difference in development of head furnishings (comb and wattles are a little larger in cocks). In

comparison with common fowls the guinea fowl feathers are of hen-like type, having short and rounded ends. This fact raises the question whether the sexual uniformity in feathering in guinea fowl is of the same nature as that of the Sebright bantams according to Morgan's findings.¹ Finlay² reported 2 castrations of guinea fowl cocks, with negative results. In the present study both cocks and hens were castrated, and resulting changes in head furnishings and plumage carefully measured.

Five cocks and 5 hens (same stock) were castrated at the age of 5 months on September 19, 1930. One week after castration certain feathers of left neck, back and tail were pulled out; corresponding feathers on the right side served as control, and after 5 months the animals were killed. Complete castration had been obtained in 3 cocks and one hen. In one cock small spherical regenerates on both sides, and in another cock a similar regenerate of testicular tissue on the left side, were found (0.05-0.1 g.). One hen regenerated on the left side an ovarian tissue with 7 macroscopically identifiable follicles (weight 0.0456 gm.); a second hen had a spherical regenerate with typical testicular tissue containing spermatozoa (sex reversion)*; a third hen showed a tissue histologically resembling an embryonic sexually indifferent gonad; the fourth hen had an hypertrophied right ovary (weight 0.0255 gm.), likewise showing the structure of a sexually indifferent gonad.

The removed plumage, regenerated in all cases in 1 month, showed no change in the shape, pigmentation and pattern even in complete castrates. Head furnishings were compared with measurements obtained on 5 normal cocks and 5 normal hens from the same stock and age. Proportions of the comb were not different in normal and castrate cocks. Hens with ovarian regenerates had only slightly smaller combs than the completely castrated female. The comb, therefore, like the plumage, is developmentally independent of the gonads.

The wattles are smaller than in control, but here also there is no pronounced difference between the completely and incompletely castrated animals. Average percentage reductions of the dimensions of the wattles of the castrates were:

	Length %	Breadth %
Cocks	-22.85	-29.94
Hens	-23.46	-21.06

¹ Morgan, T. H., *Carnegie Inst.*, 1919, 285.

² Finlay, G. F., *Brit. J. Biol.*, 1925, 2, 439.

* A detailed description of this case will be given separately.

A diminution of wattles occurs in the castrates of both fowls and guinea fowls. We think that the non-influence of castration on comb size in guinea fowls, in contrast with the diminution found in common fowls, is explained by an anatomical difference in the nature of the combs in guinea fowl and in *Gallus*. In the latter the comb consists mainly of connective tissue,³ while in guinea fowl this is mainly a spongy bony tissue which gives no response to gonadal hormones.

The sexual differentiation of the plumage in various poultry can therefore be arranged in 3 types: 1. *Primary sexual uniformity* (guinea fowls, pigeons,⁴ turkeys). Pure "hen-like" plumage, independent of gonads, of genotypical nature. 2. *Sexual dimorphism* (*G. domesticus*, ducks, pheasants, etc.). A richly differentiated, colored "cock-like" plumage, depressed in females by ovarian hormone to a "hen-like" plumage. 3. *Secondary sexual uniformity* (Sebright bantams). Here, as in group 2, genotypically a richly differentiated, colored and "cock-like" plumage which, in both sexes, becomes depressed to a uniform hen-like type by hormones of ovary and testis.

One may also consider these three types of sexual differentiation in fowls as different degrees of phylogenetic evolution.

³ Champy, Ch., and Kritsch, N., *C. E. Soc. Biol.*, 1925, **92**, 683.

⁴ Lipschütz, A., and Wilhelm, O., *C. E. Soc. Biol.*, 1928, **99**, 691.

Southern Section.

Tulane University, January 29, 1932.

6021

The Incidence of *B. Dysenteriae* in the Normal Individual.*

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In recent years mild epidemics and a few isolated cases of bacillary dysentery have been reported from various sections of the Southern States (Boyd,¹ Silverman and Harris,² Davidson³). However, these publications have dealt primarily with the active disease. There is a notable lack of information concerning the "carrier" or the occurrence of the specific bacilli of dysentery in the apparently healthy individual of this section of the country. It was, therefore, thought that a systematic study of the stool from healthy persons might prove of interest.

Fifty healthy individuals, with no history of dysentery or intestinal disturbances, were selected from various sections of New Orleans. Fresh specimens of feces, without previous purgation, were obtained through a proctoscope, and cultured immediately upon suitable media for the recovery of dysentery bacilli in accordance with the methods originally suggested by Duval.⁴ Only one stool specimen was cultured in each instance. A large number of plate colonies were transferred and studied biologically. Identification of *B. dysenteriae* was made by means of the cultural (sugars) and serological methods. It is possible that repeated bacteriological examinations might have influenced the results; however, as all cases were handled alike, it was not deemed necessary to repeat the stool examinations.

Specimens of blood were also obtained from each case, and a

* Aided by a grant from the David Trautman Schwartz Research Fund.

¹ Boyd, *Texas State J. Med.*, **17**, 261.

² Silverman and Harris, Unusual and Common Forms of Bacterial Dysentery Observed in the South. *Trans. of A. G. E. Assn.*, in press.

³ Davidson, *J. Exp. Med.*, 1920, **32**, 651.

⁴ Duval, *Am. Med.*, 1902, **4**, 417.

routine microscopic and macroscopic agglutination test carried out with stock cultures of *B. dysenteriae*, namely, Flexner, Shiga, Hiss "Y" and Duval's⁵ lactose fermenter, which was reported by him in 1904 and later confirmed by Sonne⁶ in those instances where dysentery bacilli were isolated from the stools of the normal individuals, they were tested for agglutination by the serum of the homologous case and immune serum from the experimental animal.

The following charts give the results of the first 50 cases examined:

CHARTS SHOWING REACTION OF VARIOUS STOCK CULTURES OF *B. DYSENTERIAE* AND THE HOMOLOGUS ISOLATION AGAINST THE HOMOLOGUS SERUM.

	1-25	1-50	1-100	1-200	1-300	1-400	1-600	1-800
1 <i>B. Hiss Y</i>	—	—	—	—	—			
<i>B. Duval</i>	x	x	x	x	x		x	
<i>B. Flexner</i>	x	x	—	—	—			
<i>B. Shiga</i>	—	—	—	—	—			
<i>B. dysenteriae</i> Isolated								
Duval Type	—	—	—	—	—			
2 <i>B. Hiss Y</i>	—	—	—	—	—			
<i>B. Duval</i>	x	x	x	x	x			
<i>B. Flexner</i>	x	x	—	—	—			
<i>B. Shiga</i>	—	—	—	—	—			
<i>B. dysenteriae</i> Isolated								
Duval Type	—	—	—	—	—			
3 <i>B. Hiss Y</i>	—	—	—	—	—			
<i>B. Duval</i>	x	x	x	x	—			
<i>B. Flexner</i>	—	—	—	—	—			
<i>B. Shiga</i>	x	x	—	—	—			
<i>B. dysenteriae</i> Isolated								
Duval Type	x	—	—	—	—			
<i>B. dispar</i> —Duval Type	—	—	—	—	—			
4 <i>B. Hiss Y</i>	—	—	—	—	—	—		
<i>B. Duval</i>	—	—	—	—	—	—		
<i>B. Flexner</i>	x	x	x	—	—	—		
<i>B. Shiga</i>	—	—	—	—	—	—		
<i>B. dysenteriae</i> Isolated								
Typical Duval Type	—	—	—	—	—	—		—
5 <i>B. Hiss Y</i>	—	—	—	—	—			
<i>B. Duval</i>	x	x	x	—	—			
<i>B. Flexner</i>	x	—	—	—	—			
<i>B. Shiga</i>	x	x	x	x	x			
<i>B. dysenteriae</i> Isolated								
Shiga Type	x	x	x	x	x			
6 <i>B. Hiss Y</i>	—	—	—	—	—			
<i>B. Duval</i>	x	x	x	x	x			
<i>B. Flexner</i>	—	—	—	—	—			
<i>B. Shiga</i>	x	x	x	x	x			
<i>B. dysenteriae</i> Isolated								
Duval Type	—	—	—	—	—			

x positive agglutination reaction.

— negative.

⁵ Duval, *J. Am. Med. Assn.*, 1904, **46**, 381.

⁶ Sonne, *Giftfattige Dysenteribaciller*, Kjobenk, 1914, **8**, 23.

CHART SHOWING PERCENTAGE AGGLUTINATION OF STOCK *B. DYSENTERIAE* WITH SERUM FROM FIFTY NORMAL PERSONS.

	1-25	1-50	1-100	1-200	1-300	1-400	1-600
	%	%	%	%	%	%	%
<i>B. Duval</i>	76	72	64	52	40	16	8
<i>B. Flexner</i>	64	48	28	16	10	4	2
<i>B. Shiga</i>	30	16	10	10	6	4	4
<i>B. Hiss Y</i>	6	2	2				

As shown in the charts, *B. dysenteriae* was isolated from 6 cases (12%). Of these 6, one (No. 5) was definitely of the Shiga type; 2 (No. 4 and No. 6) were Duval's lactose fermenters. The remaining 3 were also lactose fermenters, but possibly atypical forms of the Duval strain. They also correspond in some respects to the "A" and "B" Metadysentery of Castellani.⁷ In one case (No. 3), an organism, *B. dispar* (Andrew) was isolated. Ford⁸ regards *B. dispar* as belonging to the group originally described by Duval. It is interesting to note in case No. 4 in which a definite Duval strain was isolated, neither the isolated bacillus nor the stock culture of "Duval" was agglutinated, yet Flexner was agglutinated in dilution 1-100. Whereas, in case No. 5, in which the Shiga type was isolated, there was a definite agglutination of the stock Shiga culture and also the isolated organism itself in dilution 1-300, and slight agglutinations only with Duval and Flexner types. In case No. 6 the Duval organism isolated was not agglutinated, yet stock cultures of Shiga and Duval were agglutinated in 1-200 dilutions. In the remaining cases, in which borderline dysenteric organisms were recovered, only No. 2 and No. 3 agglutinated the isolated organisms.

Since the primary purpose of this work was to determine the occurrence of dysentery bacilli in the healthy individual, no attempt will be made here to explain the high percentage of agglutination of the various stock cultures of *B. dysenteriae* with the blood of these normal persons (see charts). It is recognized that there are often difficulties attendant upon the identification of strains of *B. dysenteriae* through their agglutinability with bloods of the patients; however, this means was not in any instance solely relied upon to identify the recovered cultures herein reported. Strong agglutinating sera obtained from the experimental animal and the sugar fermentation tests were commonly resorted to.

Comments. It would appear from the study of this series of cases (50) that *B. dysenteriae* occurs in the stool of the healthy in-

⁷ Castellani, Manual of Trop. Medicine. William Wood Co., N. Y. 1910.

⁸ Ford, Textbook of Bacteriology. W. B. Saunders Co., Philadelphia and London. 1927, 536.

dividual of New Orleans in approximately 12%. In a future publication it will be shown what number of these cases are true "carriers", and in what number the dysentery bacillus is a transitory sojourner of the intestinal tract. Such problems are of considerable practical importance from the standpoint of the spread of bacillary dysentery. It is noteworthy that only the lactose fermenting Duval type of *B. dysenteriae* was recovered from the normal stools in all instances except one, in which the true Shiga type was recovered. It is further noteworthy that in none of the normal stools examined was the Flexner type encountered.

6022

Susceptibility, Resistance and Spontaneous Recovery in Dogs
Experimentally Infected with *Endamoeba histolytica*.*

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In previous communications^{1, 2, 3} the writer has demonstrated a method whereby dogs may become infected with canine and human strains of *Endamoeba histolytica*. He has also proved that the dog is a particularly appropriate animal for study of amebic enteritis, since it is readily infected and manifests all of the symptoms described for human intestinal infections with this organism. Moreover the lesions in the large bowel are similar to those found in man. The present paper is based on an analysis of the protocols of 77 dogs which have been inoculated with the pathogenic ameba and on which careful observations have been made.

The essential data are set down in Table I. Eleven strains of the organism have been used, 2 of canine origin and 9 of human origin. There is also evidence that 8.4% of the positive cases were detected *post mortem* and that 12.7% of the animals which were positive at one time or another during their life failed to show any gross or histological *post mortem* evidence of infection.

The great majority of these animals were inoculated by the intra-

* Aided by a grant from the David Trautman Schwartz Research Fund.

¹ Faust, E. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 908.

² Faust, E. C., *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

³ Faust, E. C., *Am. J. Trop. Med.*, 1932, **12**, 37.

TABLE I.
Experimental canine amebic enteritis, in strains A, B (canine) C to L (human).

	A	B	C	D	E	F	G	H	I	K	L	Totals
(1) Total	53	5	2	1	1	1	1	1	1	1	10	71+6x
(2) Total positives as determined by <i>antemortem</i> tubal exam.	48	5	1	1	1	0	0	1	1	1	8	67 (87)%
(3) Total positives at necropsy	46+2x	4	2	1	1x	0	1	1x	0	0	6+2x	60 (84.5)+6x
(4) Positives not detected <i>antemortem</i>	4	0	1	0	—	0	1	—	0	0	0	6 (8.4)
(5) Death due primarily to amebic enteritis	30	3	2	0	—	0	1	—	0	0	2	38 (53.5)
(6) Death due primarily to intercurrent infections	21	2	0	1	—	1	0	—	1	1	6	33 (46.5)
(7) Cases of apparent spontaneous recovery	5	1	0	0	—	1	0	—	1	0	1	9 (12.7)
(8) Total cases positive	52	5	2	1	1	0	1	1	1	1	8	73 (94.7)

x indicates animal still living.

rectal tubal method.² Eight received the inoculum in the form of cysts by mouth. The number of inoculations utilized and the relative proportions of "takes" by each route is set down in Table II.

TABLE II.
Relationship of route of inoculation and number of inoculation to infection.

	Rectal Route		Oral Route		Total		
	+	—	+	—	+	—	%
Single inoculation	49	2	7	1	56	(72.7)	3 (3.9) 59
Double inoculation	12	0	—	—	12	(15.6)	0 — 12
Multiple inoculation	3	1	—	—	3	(3.9)	1 (1.3) 4
Natural infection	—	—	2	0	2	(2.6)	0 — 2
Total	64	3	9	1	73	(94.7)	4 (5.3) 77

TABLE III.
Primary cause of death in cases of canine amebic enteritis.

	No.	%
Amebic enteritis	38	53.5
Bronchial or lobar pneumonia	8	11.3
Intestinal bacteremias	5	7.0
Hookworm infection	5	7.0
Peritonitis	2	2.8
Demodex dermatitis	2	2.8
Ruptured gall bladder	1	1.4
Acute intestinal obstruction	1	1.4
Intussusception	1	1.4
Proctitis of undetermined origin	1	1.4
Hookworm and stomatitis	1	1.4
Hookworm and dirofilarial obstruction of right heart	1	1.4
Unknown causes	3	4.2
Sacrificed by killing	2	2.8
Total	71	100.0

It is evident that a single inoculation is usually sufficient (in 72.7% of the cases) to produce infection.

Slightly more than half of the dogs died of a primary amebic enteritis. The intercurrent diseases which were primarily responsible for the death of the other animals are indicated in Table III. However, it would be erroneous to assume that similar infections did not play an important rôle in those succumbing to amebiasis, since it is hardly possible to obtain an animal from the streets without one or more pathogenic infections.

On the basis of these data it may be stated that experimentally 94.7% of the dogs in our Tulane series have been found susceptible to infection. Theoretically it would seem that all dogs with a similar history are susceptible. Resistance in these animals is relatively low, particularly when the active inoculum is introduced by rectum into the posterior ileum. Fifty-six members of the series became infected after a single inoculation. Only a single member of the group remained uninfected after multiple attempts to produce the infection. Another dog became positive only after 5 inoculations had been made. Spontaneous recovery, in so far as it can be determined at necropsy, was indicated in 9 of the 77 cases in the series. The protocols for some of these cases give a history of acute amebic dysentery following inoculation; in others the lesions were few, there was no symptomatology, and the organisms almost immediately disappeared from the bowel. In 2 such cases, however, lesions were found *post mortem* in the subcutaneous tissues, where foci of amebae, completely walled off from the lumen of the bowel, were actively multiplying. Nevertheless, it is believed that in some of the animals, the amebae had been completely evacuated from the bowel and that recovery had been spontaneously effected.

6023

Failure to Produce Filterable Forms of the Common Pathogens in the Kendall Media.

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Kendall¹ described a culture medium containing unaltered, or nearly unaltered protein, and without peptone or significant amounts

¹ Kendall, Arthur I., *Northwestern Univ. Bull.*, 1931, **32**, 5.

of other nitrogenous substances, upon which he was able to cultivate a filterable coccus from influenza patients and filterable forms of such organisms as *B. typhosus*, *B. paratyphosus* A, *S. aureus*, *Leptospira icterohemorrhagiae*, and Dochez' scarlet fever streptococcus. We have endeavored to repeat his work using the Kendall media with *B. typhosus*, *B. diphtheriae*, *S. albus* and *S. viridans*.

Plain Kendall (K) medium sterilized by autoclaving and the clear K medium sterilized by passage through a Seitz filter after a preliminary heating to 50°C., as directed by Kendall,² were employed in the experiments. The powdered dried pig intestine was obtained by one of us (Craig) from Major J. S. Simmons, of the Army Medical School, Washington, D. C.

Cultures were made in series in both types of media, using the organisms mentioned. Incubation at 37°C. was continued for 3 days when subcultures were made. The original cultures were kept at plus 4°C. All of the cultures of each series were assembled at the end of the 5th transplant and the entire quantity of cultures of each organism filtered through newly purchased Berkefeld N filter candles. The filtrate was inoculated into double strength veal bouillon in quantities of from 0.1 cc. up to 50 cc. and incubated for several days in an attempt to elicit growth. Ten cubic centimeter quantities were also inoculated into the "plain K" medium which was incubated for 3 days and transplants made into ordinary media. In no instance did a growth of any of the 4 test organisms result in any of the cultures.

A second similar series, using the same test organisms, were filtered through Seitz filters with similar results.

The growth of these test organisms in both the Kendall "plain" and "clear" media was rather poor. The number of bacteria, in all except the cultures of *S. albus* gradually diminished, while the fifth subcultures of *B. typhosus* and *S. viridans* apparently died and could not be transplanted to either fresh Kendall or ordinary media. Living organisms survived in earlier cultures held at (+)4°C. Examination of the stained smears of the various organisms from the cultures did not reveal any unusual diminution in size other than would be expected in any poor medium which does not favor a luxuriant growth of the particular organism.

² Kendall, Arthur I., *Northwestern Univ. Bull.*, 1931, **32**, 8.

6024

Observations on the Use of Kendall's Medium in the Cultivation of *Endamoeba histolytica*.

CHARLES F. CRAIG. (Introduced by F. M. Johns.)

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The description by Kendall^{1, 2} of media containing unaltered protein and no peptones, meat extractives, or protein degradation products, thus approximating the living tissues of the body, led to an attempt to cultivate upon these media the pathogenic *Endamoeba histolytica*. This amoeba, well known to be a tissue parasite, can be cultivated in several media, and even in a simple mixture of normal salt solution and human blood serum. However, the parasite does not grow indefinitely in the more simple media that have been developed and it was thought that in the Kendall media the organism might find conditions more like those in the living tissues and that these media would prove suitable for cultivation.

A strain of *Endamoeba histolytica* originally isolated from a human case of amebiasis and which has been perpetuated in dogs was used in the experiments. Both the "plain" Kendall and the "clear" Kendall media were used, the media being planted with material obtained from the intestine of the dog containing numerous motile amebae. At the same time control cultures were made upon the Boeck-Drbohlav medium which is routinely used in the cultivation of *Endamoeba histolytica*.

The cultures were examined every day and transplants were made every 48 hours. It was noted that even after the first 48 hours the amebae were reduced in number and that after one or, at most, 2 transplants, they entirely disappeared. The cultures in the control tubes upon the Boeck-Drbohlav medium showed marked increase in the number of the amebae at the time of the first transplant and these cultures were transplanted 8 times at 48 hour intervals, at the end of which time the organisms were still present in the usual number found in cultures upon this medium.

Further observations will be made upon this subject but it is evident that the unmodified Kendall media are not suitable for the cultivation of *Endamoeba histolytica*.

¹ Kendall, Arthur I., *Northwestern Univ. Bull.*, 1931, **32**, 5.

² Kendall, Arthur I., *Northwestern Univ. Bull.*, 1931, **32**, 8.

Occurrence and Significance of Certain Strains of *B. Dysenteriae*
in Bacillary Dysentery for New Orleans Area.

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While it is well known that many of the dysenteric infections of the far South are caused by *Endamoeba histolytica*, there undoubtedly are a considerable number of cases in which this protozoon has no etiologic relationship. In such cases, we find some one of the strains of *B. dysenteriae* playing the sole causal rôle. It was first suggested to Doctor C. W. Duval by Doctor Simon Flexner (personal communication) that the bacillary dysentery incidence should be determined for the New Orleans area, particularly as this region presents at all times many cases of amebic colitis.

Material for this study was obtained from approximately 100 sporadic cases of dysentery in which no protozoa or other animal parasites were detected in the dejecta. The clinical aspects of these cases have been previously reported.¹ The group comprises acute, subacute, and chronic forms of the disease. The specimens, with few exceptions, were fresh bowel scrapings obtained directly by means of proctoscopic examination. Fletcher and Jepps² pointed out that material for bacteriologic studies obtained in this manner is far more desirable than the stool, because the likelihood of culturing the causal agent is greatly enhanced. Plating of the fresh mucosal scrapings was carried out in the usual way and suspected colonies of *B. dysenteriae* transferred to differential culture media. The recovered dysentery cultures were further identified with respect to strains and then tested with homologous immune sera and the serum from the particular case to determine etiologic relationship.

Bacteriologic investigations into the cause of acute infectious dysentery have been thoroughly studied for epidemic outbreaks in the United States by Flexner and his coworkers,³ and distinct strains

¹ Silverman, Daniel N., and Harris, Wm., *Trans. Am. Gastro-Enterol. Assn.*, 1931, in press.

² Fletcher, William, and Jepps, Margaret W., *Studies from the Institute for Medical Research, Kuala Lumpur, Fed. Malay States*, No. 19. John Bale, Sons and Danielson, Ltd., London, 1924.

³ Flexner, Simon, and co-workers, *Studies from the Rockefeller Institute for Medical Research*, 1904, 2.

of the Shiga bacillus have come to be recognized. Duval⁴ reported the finding of another strain of the Shiga group which differed from the strains previously identified in that it fermented lactose. The findings of Sonn ⁵ and of Castellani⁶ confirmed those of Duval. While Sonn  gives due credit to Duval for first pointing out that a lactose fermenter causes bacillary dysentery and was the outstanding strain in the cases reported by him, subsequent writers, particularly Nelson,⁷ incorrectly refer to the lactose fermenter as the Sonn  bacillus.

In my series, several strains of *B. dysenteriae* were isolated and identified from the cultures prepared from the fresh mucosal scrapings. The great majority (60-65%) were of the Flexner type. Next in number were the lactose fermenter of Duval. Not more than 3% were of the true Shiga strain. It is noteworthy that the Hiss-"Y" strain was not encountered. One case yielded the strain known as the Flexner-Harris type of organism, which was agglutinated only by the blood serum of the patient from whom the organism was isolated. The various strains of *B. dysenteriae* recovered reacted significantly with their homologous sera and, in lower dilutions, cross agglutination reactions were also obtained.

Strains of *B. dysenteriae* Isolated from 100 Cases of the Series.

B. Flexner	75	B. Shiga	4
B. Duval	20	B. Flexner-Harris	1

From my series the prevailing type of bacillary infection is due to the Flexner strain of the dysentery bacillus. On the other hand, it is significant that, for the relatively small number of cases studied, many of them yielded the lactose fermenting bacillus of the dysentery group. They were not only recovered from adults but from children and infants. This is of interest because it indicates that the lactose fermenter also plays an etiologic r le in infantile diarrhea. The recovery of the Shiga type of organism in so few of my cases is consistent with the findings of others elsewhere in respect to sporadic bacillary dysentery.

⁴ Duval, C. W., *J. Am. Med. Assn.*, 1904, **46**, 381.

⁵ Sonn , C., *Zentralb. f. Bakt. I., Abt. Orig.*, 1915, **75**, 408; **76**, 65.

⁶ Castellani, Aldo, and Chalmers, A. J., *Manual of Tropical Medicine*, William Wood and Co., New York, 1910.

⁷ Nelson, R. L., *J. Bacteriol.*, 1930, **20**, 183.

Iowa Section.

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6026

Physico-Chemical Mechanism of Cellular Electromotive Force.

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The physico-chemical nature of the intracellular electromotive system is not deducible from electromotive force measurements alone, although many investigators have drawn conclusions from such data. Critical evidence has been provided by Lund and his students¹ that the E.M.F. arises from an oxidation-reduction system. This, and direct evidence by the writer,² are interpretable only in terms of a conductor of the first class (plasma membrane, or analogous structure or phase boundary) in electron equilibrium with a dissolved ion or ions. Under these circumstances the effect of electric currents upon cells acquires diagnostic significance. Mast³ has recently confirmed Kühne's observation that passage of current through the epidermal cells of *Tradescantia* causes acidity at the cathode end and alkalinity at the anode end. The cells contain a natural pH indicator; the color change begins at the inner boundary of the cell membrane and extends slowly inward. The writer has also confirmed these experiments. The color change is a *qualitative test* of the nature of the electromotive system.

Four physico-chemical systems must be interrogated as to their behavior on electrolysis (the diffusion potential will not explain the facts in *Tradescantia*). The membrane potential and the phase boundary may be treated together under the Donnan equilibrium. In this system electrolysis causes accumulation of the non-diffusible ion at one membrane and diminution at the other. The non-diffusible ion tends at equilibrium to expel ions of like signs and to attract in ions of opposite sign. If the ion is an ampholyte, *e. g.*, a

¹ For references consult Lund, E. J., *Protoplasma*, 1931, **13**, 236; *Plant Physiol.*, 1931, **6**, 631.

² Marsh, G., *Protoplasma*, 1930, **11**, 447.

³ Mast, S. O., *Z. für Vergleich. Physiol.*, 1931, **15**, 309.

protein, then on the acid side of the isoelectric point the interior will be alkaline to the exterior; increase in concentration of non-diffusible ion will cause an increase in internal alkalinity, decrease in concentration, an increase in acidity. On electrolysis the non-diffusible ion will accumulate at the cathode membrane, increasing the internal alkalinity; it will diminish at the anode membrane, causing increase in acidity. On the alkaline side of the isoelectric point increase of non-diffusible ion will cause increased internal acidity; this will occur at the anode membrane on electrolysis. The reverse will occur at the cathode membrane. These pH changes can be demonstrated by passing a current through gelatin salts between collodion membranes, using appropriate indicators. Exactly the opposite changes are observed in *Tradescantia*. A Donnan equilibrium (while probably existing) is not the electromotive system responsible for this phenomenon.

If the mobility of H^+ or of OH^- ions were decreased in the membrane the color changes in *Tradescantia* would be accounted for. In this event the exterior of the anode membrane should become acid, the exterior of the cathode membrane alkaline. Injured or dead cells occur scattered through the epidermis on both sides of living cells, the dye showing the color corresponding to the pH of the external medium (usually alkaline). Prolonged current flow causes no color change of the dye adjoining either the anode or cathode ends of living cells. The zone of color change in living cells may be greater than 10 times the width of 2 cell walls, so that the reverse pH changes should be visible along the boundary of the adjoining cells. Evidently no marked decrease in mobility of H^+ or of OH^- ions occurs in the membrane.

The pH changes at the surfaces of conductors of the first class on electrolysis are conventionally stated to be alkalinity at the cathode surface and acidity at the anode surface. If, however, the electrolyte is an ion which readily changes valence at the electrodes and water is not decomposed, it is evident that at the cathode cations are destroyed or anions created, at the anode cations are created or anions destroyed, depending upon the sign of charge of the electromotively reversible ion. Simultaneously H^+ and OH^- ions respectively move to and accumulate at the cathode and anode. *Acid* appears at the *cathode* and *alkali* at the *anode*. This may be nicely demonstrated by electrolyzing $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ between platinum electrodes with phenol red.

A conductor of the first class in electron equilibrium with a dissolved ionic species capable of ready valence change is the only

physico-chemical system adequate to explain the pH changes in *Tradescantia*. The conclusion seems inescapable that this is the type of system found in the living cell, for the mechanism of production of E.M.F. is probably common to all forms of living matter. That the cell surface is both a "membrane" and a "solid" conductor of electrons is no real paradox.

6027

The Demonstration of *Mycobacterium Tuberculosis* in Exudates, Tissues and Body Fluids.

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Five hundred and twenty-seven parallel attempts were made to recover the *Mycobacterium tuberculosis* from various materials taken from lesions suspected of being tuberculous, using guinea pig inoculation and Corper's gentian violet-glycerol-water-potato slants.

TABLE I.

	Tissue	Urine ureteral	Urine voided	Urine bladder	Exudate	Spinal fluid	Sputum	Stool	Gastric content	Cyst fluid	Total
Pig neg.	48	202	10	10	70	15	5	2		1	363
Cult. neg.											
Pig pos.	15	17	2		10	3			1		48
Cult. pos.											
Pig pos.	9	4			11	3			1		28
Cult. neg.											
Pig pos.		2		1	1						4
Cult. inc.											
Pig neg.	3	2	1		1						7
Cult. pos.											
Pig inc.	4				1						5
Cult. pos.											
Pig neg.	2	13		2	5		1				23
Cult. inc.											
Pig inc.	9	20		3	8		3				43
Cult. neg.											
Pig inc.	1	1	2		1					1	6
Cult. inc.											
Total	91	261	15	16	108	21	9	2	2	2	527
Agreement, positive	48										12
Agreement, negative	363										32

Pig superior:		Culture superior:	
Pig positive, culture negative	28	Culture positive, pig negative	7
Pig positive, culture incomplete	4	Culture positive, pig incomplete	5
Pig negative, culture incomplete	23	Culture negative, pig incomplete	43
	55		55

The material for seeding on the medium was at first treated with 6% sulphuric acid and later with 5% oxalic acid when Corper reported more favorable results with the latter method. The treatment in either instance was for 30 minutes at 37°C. Cultures were incubated for 12 weeks, and guinea pigs were killed in 6 weeks if they had not already died. Failure to complete the guinea pig tests was due almost entirely to death by secondary infection before tuberculous lesions had opportunity to develop. Failure to complete the culture tests was divided between loss by breakage in the centrifugalization, overgrowth by fungi or spore forming organisms, each 8 times, and 7 instances in which the material was treated for a longer period of time than that recommended and hence the negative results were discarded.

It is concluded that, from the standpoint of a diagnosis of tuberculosis, the guinea pig method surpasses the culture as a routine procedure.

6028

The Rôle of Proteins in Growth, Reproduction and Lactation. II. The Influence of Whole Liver on Growth and Lactation.*

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It was reported¹ that second generation albino rats on 2 diets, A and B, containing 15 and 20% liver protein respectively, were unable to nurse their young. The failure of lactation was not caused by a deficiency of vitamins since the administration of increased amounts of yeast and cod liver oil as well as the daily administration of 0.3 cc. of wheat germ oil led to no improvement. The daily administration of 0.5 to 1.0 gm. of dried whole liver (dried in a vacuum at 100°C.) also had no beneficial effect on lactation, where-

* This work was in part supported by a grant from the National Live Stock and Meat Board.

¹ Smith, H. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 597.

as 1.5 gm. of raw liver daily enabled the animals (9) to nurse their young successfully, 26 animals (45% of the 57 born) being weaned at 21 days of age. The success of raw liver in imparting the ability to lactate is further demonstrated in that third generation animals (2 males and 4 females on each diet) were considerably heavier than the parent generation had been at the same age, the males remained fertile longer than the second generation males, the females had normal oestrus cycles and they were able to nurse their young successfully without additional supplement to the diet.

Fifteen first generation animals reared on the diets supplemented by 0.5 gm. of dried whole liver in addition to yeast and cod liver oil grew at a faster rate than 32 control animals. Lactation was also improved by the dried liver supplement in that the percentage of the young which were weaned was approximately twice that of control animals (45% as against 26%) and the young were considerably heavier when weaned. The influence of the whole liver does not reside in the added protein or fat since growth and lactation were not appreciably influenced by supplementing the diets of a like number of animals with fat-free liver or liver fat equivalent in amount to the dried whole liver supplement.

6029

Attempts to Immunize Rabbits Against a Larval Cestode,
Cysticercus Pisiformis.*

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From the Department of Zoology, Washington University.

Cysticercus pisiformis is the larval form of *Taenia pisiformis* (*serrata*), a tapeworm found in the intestine of the dog. The larvae begin development in the liver of the rabbit and later migrate to the mesenteries, where they form typical cysticerci. Rabbits were injected with *Taenia pisiformis* material and subsequently fed with onchospheres of this species. Two cc. of a 1% suspension of fresh or powdered worm material in physiological saline solution were injected intraperitoneally, subcutaneously, or intravenously at intervals of 2 or 3 days for a total of 6 injections. From 3 to 19 weeks after the last injection the control and injected rabbits were fed equal amounts of a uniform suspension of mature onchospheres by

* This investigation was in large part made possible by a research grant to Washington University from the Rockefeller Foundation.

stomach tube. They were autopsied 3 to 6 weeks later, during the period when the larvae were migrating from the liver to the mesenteries in the control animals.

Development of the larvae was totally inhibited in a few of the rabbits which had received injections of adult worm material. In most instances the difference in the condition presented by the livers of the control and injected animals was one of degree; in only a few cases were larvae found to be migrating in the experimental animals; while in the control rabbits migration of the larvae frequently occurred or the larvae in the liver were larger than those in the rabbits which had received injections. The data were derived from 73 animals, of which number 34 were controls. The results of one experiment are shown in Table I.

TABLE I.
Number of cysts and migrating larvae present in liver at autopsy.

Injected Rabbits	Total Larvae	Living Cysts		Migrating Larvae	Small Dead Cysts
		Large	Small		
42	3	3	—	—	—
44	14	2	6	—	6
40	15	7	16	—	2
41	28	—	10	—	18
43	30	23	7	—	—
Aver.	20	7	6	—	5
Controls					
47	26	18	7	1	—
48	27	15	11	—	1
49	38	3	21	—	14
38	42	35	3	4	—
46	58	46	12	—	—
Aver.	38	23	11	1	3

A number of animals were found to have been infected previous to purchase. When this occurred among the controls, some evidence of a degree of protection against superinfection was observed.

6030

Effect of Adrenal Cortex Extract on the Course of Certain Human Infections.

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(Introduced by Ivan E. Wallin.)

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Much experimental evidence indicates that the adrenals possess a detoxicating function. Lewis¹ showed that double adrenalectomy increased the sensitiveness of rats to cobra venom, adrenalin and diphtheria toxin. Scott² demonstrated the greatly reduced resistance of adrenalectomized animals to doses of killed streptococci and staphylococci. Belding and Wyman³ confirmed Lewis' work that adrenalectomy lowers the resistance of animals to diphtheria toxin. Recently several investigators have found increased resistance to bacterial intoxication is conferred by a cortical adrenal extract.⁴ Scott and Bradford⁵ found that the extract seemed to decrease slightly the susceptibility of adrenalectomized rats to killed typhoid bacilli. Hartman and Scott⁶ later demonstrated that the resistance of adrenalectomized rats to bacterial intoxication was significantly increased by adrenal cortex extract.

These findings prompted us to use injections of cortical adrenal extract in patients with bacterial intoxication in the Colorado General Hospital. A preparation was made according to the technic of Swingle and Pfiffner and contained the equivalent of 50 gm. gland per cc. Injections were usually given intramuscularly. The first case treated was a typical typhoid fever. The diagnosis was confirmed by blood culture, urine culture and Widal reaction. Two cc. of the extract were given on the eleventh day after the first prodromal symptoms. The temperature became normal on the second day following injection and remained so during the 14 days in the hospital.

The second case was one of undulant fever of 6 weeks' duration. Diagnosis was confirmed by blood culture and agglutination tests.

¹ Lewis, *Am. J. Physiol.*, 1923, **64**, 506.

² Scott, *J. Exp. Med.*, 1924, **39**, 457.

³ Belding and Wyman, *Am. J. Physiol.*, 1926, **78**, 50.

⁴ Swingle and Pfiffner, *Science*, 1930, **72**, 321.

⁵ Scott and Bradford, *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 428.

⁶ Hartman and Scott, *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 478.

Two days following the injection of 2 cc. cortical extract the patient's temperature returned to normal and remained so for one week, when it showed an afternoon rise which promptly subsided following a second similar injection. The temperature remained practically normal during the rest of his stay in the hospital (9 days), only rising on 2 occasions to 99.2°F.

The third case was postoperative erysipelas with a positive blood culture which showed marked improvement after intramuscular injection of 2 cc. cortical extract. The organisms disappeared from the blood stream but later a relapse set in. The blood culture became positive and the patient died during an acute asthmatic attack before a second injection of cortical extract could be given.

The fourth case was one of lymphangitis of the hand and arm which had improved under incision and drainage but later developed a high fever. A blood culture taken at this time was positive, showing *Micrococcus aureus*. The temperature promptly returned to normal and remained so after one injection of cortical hormone.

The fifth case, a girl of 19, complained of chills, cough, nausea and vomiting, 12-11-1931. Physical examination was essentially negative. The temperature ranged between 102° and 106°F. Her condition did not improve and on December 23rd, 4 cc. of cortical adrenal extract were given hypodermically and the same dose repeated the following day. On December 25th she was much improved and no extract was given. On December 26th her fever was again high and 4 cc. of adrenal extract were administered. This dose was repeated on December 27th. Two cc. of cortical extract were given daily for the next 4 days. She improved rapidly following this and was discharged on January 16th. The diagnosis was a virulent ethmoid sinus infection, peribronchial adenitis with possible mesenteric adenitis.

While definite conclusions cannot be drawn from such a short series of cases, in our opinion the results are suggestive and tend to confirm our assumption that cortical adrenal extract might have some action in increasing the resistance of the human body to bacterial intoxication. Additional studies now in progress, should more definitely prove or disprove this thesis.

We are indebted to J. T. Thompson and to T. J. Becker for the supply of adrenal cortex extract used in this work.

